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EDITED BY

W. H. PEARSALL
D.Sc., F.R.S.
Quain Professor of Botany
University College, London

ASSISTED BY

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Imperial College of Science and Technology, London

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Studies in the Physiology of Obligate Parasitism. V

Further Differences between the Uredospore Germ-tubes and Leaf Hyphae of *Puccinia triticina*

BY

SYDNEY DICKINSON

(*School of Argiculture, Cambridge*)

ABSTRACT

Some physiological differences (including the study of carotin pigmentation) between the ectophytic and endophytic stages of *Puccinia triticina* Erikss. have been investigated, and it is suggested that the principal difference is one of cell-wall permeability, the ectophytic stage having a lower permeability than the endophytic stage.

INTRODUCTION

DIFFERENCES between the ectophytic and endophytic mycelia of many parasites have been observed. In *Ophiobolus graminis* the dark colour and large diameter of the runner hyphae on the outside of the infected roots are in contrast with the light colour and small diameter of the hyphae inside the root tissue. This is a striking example where, and probably in others too, the difference in nutrition is usually considered to be the determining factor.

The ectophytic stage of *Puccinia triticina* is composed of the spore, germ-tube, appressorium, and substomatal vesicle. The latter admittedly is formed in the substomatal cavity of the leaf, but is really a continuation of the appressorium (Dickinson, II, 1949). The endophytic stage is started by the first hypha—the infection hypha—formed by the substomatal vesicle, and is composed of leaf hyphae, haustorial mother cells, and haustoria. As it is not considered that there is any significant difference between the first or infection hypha and the subsequent or leaf hyphae, the latter term will be used in this paper.

The morphological similarity shown in the development of appressoria and substomatal vesicles by the germ-tubes and the development of haustorial mother cells and haustoria by the leaf hyphae has already been described (Dickinson, II, 1949). The contrast between the ectophytic and endophytic stages are summarized in Table I. The further differences between the two stages considered in this paper are the carotin pigment content, the permeability of the cell-walls, and the hydrotropic reactions.

EXPERIMENTS ON CAROTIN

The orange-red coloration, seen in germ-tubes about to form appressoria, in typical appressoria, and in the later-formed substomatal vesicles, was the most readily observed of the differences between germ-tubes and leaf hyphae,

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whether growing on the plant or on membranes. The leaf hyphae produced by substomatal vesicles, either on the plant or on most membranes, soon lost this

TABLE I

Differences between the Uredospore Germ-tubes and Leaf Hyphae of Puccinia triticina (ref. *Ann. Bot.*, N.S., xiii)

Germ-tubes.	Leaf hyphae.
Diameter about 10 μ	Diameter about 5 μ
Red colour in appressoria, &c.	No red coloration observed
No nuclear division until substomatal vesicle formation	Frequent nuclear division
No septation	Frequent septation
Bursts on occasion	No bursts observed*
Formation of appressoria and substomatal vesicles after thigmotropic stimulation	Formation of haustorial mother cells and haustoria after thigmotropic stimulation
Mesophyll cell-walls, stained by cotton blue in lacto-phenol, when in contact with germ-tubes	Mesophyll cell-walls not stained by cotton blue in lacto-phenol when in contact with leaf hyphae

* Bursts of leaf hyphae have since been observed on certain membranes.

red coloration (Table II). However, on certain membranes patches of red colour, adjacent to the septa, and on the side towards the growing-point, persisted throughout life in the leaf hyphae.

TABLE II

Observations on the Appearance and Disappearance of Red coloration (reduced Carotin) in Puccinia triticina

Type of mycelium, &c.	Position.	Conditions.	Observation.
Uredospores	—	On germination	Disappearance
Germ-tubes	In contact with membranes	On formation of appressoria, &c.	Appearance
,,	,,	On bursting	Disappearance
,,	Submerged	In water	Appearance
,,	,,	In dye solutions (e.g. Malachite green, &c.)	,,
,,	Aerial	On slight but sudden reduction in humidity	,,
Leaf hyphae	Submerged	In dye solutions (e.g. Malachite green, &c.)	,,
,,	In contact with certain membranes	On formation of septa and branches	,,
,,	,,	On bursting	Disappearance
,,	In the leaf	On spore formation	Appearance

The disappearance of the orange-red colour on the 'bursting' of the germ-tubes has been previously described (Dickinson, II, 1949). On membranes where a high percentage of bursts (50–70 per cent.) occurred, the coloration was usually more of a yellow than an orange-red tint. In such cases there was

some persistence of the yellow, but not of the red colour, after the burst. Bursting of leaf hyphae on certain membranes has been observed, and these leaf hyphae were always coloured orange-red. This colour disappeared at once when the burst occurred. A red coloration has been seen in germ-tubes growing under water, but leaf hyphae from sections of infected leaves have never developed a red colour under water. They do develop red-coloured granules when submerged in certain dye solutions. Germ-tubes usually develop a yellow colour when suddenly exposed to a lower relative humidity, but this has not been seen to occur in leaf hyphae, whether from the host or on membranes.

The appearance and disappearance of the yellow or red coloration is associated on the plant with the start and finish of the ectophytic stage, i.e. in spores and substomatal vesicles, but on membranes partly with the type of underlying membrane and partly with conditions which might suggest a deficiency or variation in available oxygen, i.e. submerged in water.

No mention has been found in the literature of any such coloration except in the spores. In *Puccinia graminis* Newton and Johnson (1927) suggested, on the basis of chemical tests, that the red coloration of the spores was due to the presence of reduced carotin. They found that acetone extracts of uredospores when treated with concentrated sulphuric acid turned a deep blue colour. This is the standard test for the presence of carotin. The orange-red colour indicated that it was in a reduced condition. In the oxidized condition the carotin pigment loses its red colour.

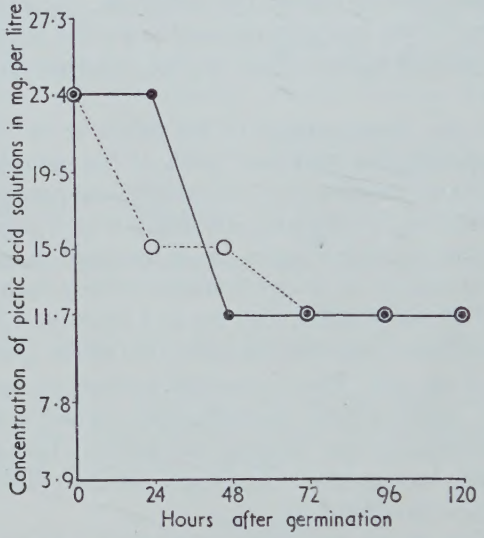
The red coloration of the uredospores was similar to that seen in perfect appressoria and substomatal vesicles, whether formed on the plant or on membranes, but that seen in the germ-tubes and atypical appressoria, &c., is more yellow in colour. In view of the similarity of colour it would seem probable that this red or yellow colour was due to the presence of the same pigment, reduced carotin. A difficulty in demonstrating this has been the presence, in about equal amounts, of carotin in the germ-tubes. Consequently a direct test of the presence of carotin was invalid.

A preliminary test showed that 5 mg. contained about 23,000 uredospores, and that by careful handling, 400–500 spores could be distributed on one membrane to give an evenly dispersed load. Each 5 mg. of spores was distributed over some 50–60 membranes. All membranes, for any one experiment, were made and used the same day. Distilled water only was placed below the membrane. Germination took place in a humidity chamber (R.H. *c.* 98 per cent.).

Two types of membrane (Dickinson, II, 1949) were used. On the first—precoloured nitrocellulose—only germ-tubes were produced, no other sign of thigmotropic reaction being seen except that of direction of growth. On the second—wax nitrocellulose—a high percentage (70 per cent.) of the germ-tubes formed appressoria, &c.

The extracts were made by grinding all membranes carrying one sample of 5 mg. of spores with a very little absolute alcohol. After grinding, the alcohol

was made up to 5 c.c. and diluted to 90 per cent. Ten cubic centimetres of petroleum ether was then added, and the mixture thoroughly shaken. The petroleum ether, after settling, was decanted off, and its colour matched with a dilution series of picric acid solutions in water. For matching, a Hellige comparator, tubes, and blanks were used. After matching, the sample was divided into two, and sulphuric acid was added to one half and trichloracetic



Relation between length of time after germination and the intensity of colour of petroleum ether extracts from 5 mg. samples of uredospores of *Puccinia triticina*, as matched against a series of picric acid concentrations. Each 5 mg. sample of spores was germinated on 50–60 membranes. The germination was over 80 per cent. throughout. No appressoria were observed on precoloured nitrocellulose membranes. The appressorial percentage on wax nitrocellulose membranes was 70 or over. Distilled water only was placed below all membranes.

acid added to the other. All extracts of spores, both ungerminated and germinated, turned blue on the addition of either acid. All control extracts of membranes without spores were colourless.

In the graph it will be seen that the extract from ungerminated spores on both precoloured and wax nitrocellulose membranes (Dickinson, II, 1949) matched with 23·4 mg./l. picric acid. After 24 and 48 hours of growth, extracts from precoloured membranes matched with 15·6 mg./l. picric acid. Wax membranes after 24 hours matched with 23·4 mg./l. picric acid, and after 48 hours with 11·7 mg./l. picric acid. All extracts from both types of membrane

matched with 11·7 mg./l. picric acid after 72 hours or more of growth. A similar result was obtained in all six repetitions of the experiment. Observation of pre-coloured membranes showed no sign of appressorial formation at any time. After 24 hours wax membranes had numerous orange-red coloured germ-tubes, appressoria, and substomatal vesicles. After 48 hours almost all the appressoria and substomatal vesicles had lost their red colour and had produced colourless, or very slightly coloured, leaf hyphae.

The germination percentage was 80 or over. Now 20 per cent. of 5 mg. of spores would mean about 4,000 spores, and extracts of about 3,000 ungerminated spores matched with 3·9 mg./l. picric acid in three different tests; consequently the high intensity of colour in the 24-hour wax membrane extracts could not have been due to the presence of possibly 20 per cent. ungerminated spores.

As the ungerminated spore and appressorial containing extracts matched with a consistently higher percentage of picric acid than all other extractions, and all extracts contained carotin, it would appear that the greater intensity of colour, as indicated by the picric acid matching, in the appressorial extracts, was due to the same cause as that of the ungerminated spores—the presence of carotin in a reduced condition.

In an attempt to provide further evidence, numerous experiments have been made to try to decrease the amount of reduced carotin in the appressoria and substomatal vesicles by providing an increased oxygen supply in one form or another, but have invariably failed. A possible hypothesis for this failure and for the appearance of reduced carotin might be that it was due to the permeability of the germ-tube or appressorial wall being low. In the next section a test of this suggestion will be described.

Experiments on Permeability

Stock (1931) made a detailed study of germ-tube growth of *Puccinia triticina* as well as other Rusts. During this study he immersed uredospore germ-tubes in a range of dyes. In the present tests a similar range of dyes has been used in two concentrations, 1/1,000,000 and 1/100,000. The stronger concentration was chosen as that at which signs of inhibition of germ-tube growth first became apparent with several of the dyes. Preliminary observations showed that the protoplasm of the appressoria and their 'bulges' appeared to stain more easily than that of the germ-tubes. Such tests were not considered reliable as the dye solutions were not in direct contact with the germ-tubes, and there was a possibility that in unstained specimens the dye might not have passed through the membrane. Consequently only germ-tubes and leaf hyphae directly in contact with the dye solutions have been used. For the former, freshly gathered uredospores were allowed to germinate on drops of the dye solutions placed on microscope slides, and for the latter, leaf strips, from which leaf hyphae had started to grow out, were laid on the dye solutions in watchglasses. All specimens were kept in a humidity chamber (R.H. *c.* 98

per cent.) except when under examination. Observations were made after 24 hours. The results of the tests, which were repeated on five separate occasions, are given in Table III. The uptake of dye by submerged germ-tubes and leaf hyphae only were recorded as the germ-tubes or leaf hyphae growing above the solutions were almost invariably unstained. The effects of the dye solutions on ungerminated spores were not considered reliable and so were not included. The percentage of germination in all tests was 80 or over.

TABLE III

Effect of submerging Germ-tubes and Leaf Hyphae of Puccinia triticina in a Series of Dye Solutions

	Concentration 1 pt. in 1,000,000		Concentration 1 pt. in 100,000	
	Germ-tubes.	Leaf hyphae.	Germ-tubes.	Leaf hyphae.
Methyl violet	–	++++	++++	++++
Malachite green	+++	++++	++++	++++
Safranin	+++	++++	+++	++++
Congo red	+++	++++	+++	++++
Basic fuchsin	++	++++	++	++++
Chrysoidine	–	–	++++	++++
Methylene blue	–	–	++	++++
Aniline blue	–	++++	–	++++
Victoria blue	–	++++	–	++++
Methylene green	–	–	–	++++
Victoria green	–	–	+	++++
Acid fuchsin	–	–	+	–
Bismark brown	–	–	–	–
Night blue	–	–	–	–
Light green	–	–	–	–
Orange G	–	–	–	–

++++ = Definite staining
+++ = Some staining
++ = Slight staining
\+ = Very slight staining
– = No visible stain

Examination of Table III shows that the protoplasm of the submerged germ-tubes—with no visible thigmotropic reactions—was stained in 1/1,000,000 solutions of safranin, malachite green, Congo red, and basic fuchsin. The protoplasm of leaf hyphae—with no visible thigmotropic reactions—was stained, not only by the same four dyes, but by three others as well at the same concentration. At the higher concentration, 1/100,000, a larger number of the dye solutions stained the protoplasm of both the germ-tubes and leaf hyphae, but the number of different dye solutions staining the leaf hyphae was again greater than that which stained the germ-tubes. Only acid fuchsin solution 1/100,000 stained the germ-tubes and not the leaf hyphae.

Germ-tubes and leaf hyphae submerged in methylene blue solutions of both strengths always contained some orange-red coloured granules. In both concentrations of safranin and malachite green solutions some of the germ-

tubes and leaf hyphae contained red granules. These red granules were seen in leaf hyphae immersed in the stronger concentrations only of Congo red and Victoria green. In basic fuchsin no red granules were observed.

To determine the time taken for the stain to appear in the protoplasm,

TABLE IV

Time required for Stain to be visible in Uredospore Germ-tubes and Leaf Hyphae of Puccinia triticina when submerged in a Series of Dye Solutions

Dye.	Time in hours.	Dye concentration: 1 in 1,000,000 Germ-tubes.	1 in 1,000,000 Leaf hyphae.	1 in 100,000 Germ-tubes.	1 in 100,000 Leaf hyphae.
Safranin	1	—	—	—	+
	2	—	—	—	+
	3	—	—	—	+
	5	—	—	—	+
	6	—	—	+	+
	7	—	+	+	+
	8	+	+	+	+
Congo red	1	—	—	—	—
	2	—	+	+	+
	3	+	+	+	+
	5	+	+	+	+
	6	+	+	+	+
	7	+	+	+	+
Basic fuchsin	1	—	—	—	+
	2	—	—	—	+
	3	—	—	—	+
	5	—	—	+	+
	6	—	+	+	+
	7	—	+	+	+
	8	+	+	+	+

Dye.	Time in hours.	Dye concentration: 1 in 1,000,000 Germ-tubes.	1 in 1,000,000 Leaf hyphae.	1 in 100,000 Germ-tubes.	1 in 100,000 Leaf hyphae.
Malachite green	1	—	—	—	+
	2	—	—	—	+
	3	—	+	—	+
	5	—	+	—	+
	6	—	+	+	+
	7	—	+	+	+
	8	+	+	+	+
Methylene blue	1	—	—	—	+
	2	—	—	—	+
	3	—	—	—	+
	5	—	+	—	+
	6	—	+	+	+
	7	+	+	+	+
Victoria green	1	—	—	—	+
	2	—	+	—	+
	3	—	+	—	+
	5	—	+	+	+
	6	—	+	+	+
	7	—	+	+	+
	8	+	+	+	+

— = No stain visible. + = Stain visible.

Difference in hours between onset of visible staining in leaf hyphae and germ-tubes, together with observations on inhibition of growth in germ-tubes

Dye.	Dye concentrations: 1 in 1,000,000.	1 in 100,000.	Inhibition observed in germ-tubes.
Safranin	1	5	Yes
Congo red	1	0	No
Basic fuchsin	2	4	Yes
Malachite green	5	5	Yes
Methylene blue	2	5	Yes
Victoria green	6	4	No

hourly examinations were made using six of the dye solutions at both concentrations. The results are shown in Table IV. Except in the stronger concentration of Congo red, the stain of all solutions appeared first in the leaf-hyphae protoplasm. The rapidity of appearance was considerably different in the various dyes.

Using Congo red and Victoria green, no inhibition of germ-tube growth was observed, and the difference in hours between the onset of visible stain in the

germ-tubes and leaf hyphae was greater at the lower concentration. With the remaining four dyes, slight inhibition of germ-tube growth was observed, and the difference in hours between the onset of visible stain in the germ-tubes and leaf hyphae was greater at the higher concentration. It would seem probable that there was a correlation between inhibition, and the time required for the stain to become visible.

These experiments showed that the permeability of the leaf hyphae was greater than that of the germ-tubes.

Experiments on Hydrotropism

A difference in the permeability of the walls of germ-tubes and leaf hyphae might well be expected to influence their behaviour to alterations of relative humidity. Previously it had been noticed that the direction of growth of the leaf hyphae formed by substomatal vesicles on membranes was usually away from the membrane, while that of the germ-tubes was along the membrane surface (Dickinson, II, 1949). The relative humidity in these experiments was *circa* 98 per cent. When a lower relative humidity was used—produced by a drop of 2·5 per cent. H_2SO_4—the direction of growth of the leaf hyphae was along the membrane. This observation suggested a difference in the hydrotropism between the leaf hyphae and the germ-tubes.

To determine the hydrotropic reactions of the leaf hyphae was difficult because these hyphae grew from among mesophyll cells, which provided a relatively large water source. However, a comparison has been made between the direction of growth of such hyphae and of germ-tubes arising from spores germinating on the same mesophyll cells.

Strips of exposed infected mesophyll were placed on agar in a modified Van Tieghem drop cell. As soon as the hyphae were about to grow out—after about 12 hours—uredospores were dropped on to the mesophyll cells. The spores had to be placed on the mesophyll cells after hyphal growth had begun, because the germ-tubes would otherwise be much longer than the leaf hyphae either owing to their higher rate of growth or to their more rapid start of growth. The humidity gradient in the atmosphere, through which the germ-tubes and leaf hyphae grew side by side, was varied by altering the concentration of sulphuric acid on the coverslip.

The results are shown in Table V. The direction of growth of the germ-tubes was altered (i.e. 'looping' had occurred; Dickinson, I, 1949) in the lower relative humidity, when the acid concentration was 5 or 10 per cent. The direction of growth of the leaf hyphae, growing alongside the 'looping' germ-tubes, was unchanged. The length of growth, as measured by eye, of both germ-tubes and leaf hyphae was lower when the higher concentrations of acid were used. This alteration in the direction of growth of the germ-tubes, and not of the adjacent leaf hyphae, considered in combination with the reduced amount of growth of both germ-tubes and leaf hyphae, demonstrated that there was a difference in their hydrotropic reactions.

TABLE V

Direction and Length of Growth of Uredospore Germ-tubes and Leaf Hyphae of Puccinia triticina in a Series of Humidity Gradients*

The leaf hyphae were grown from exposed infected mesophyll, to which uredospores were added after 12 hours. The humidity gradient was altered after 24 hours.

	First 24 hours			Subsequent 48 hours							
				Liquid above mesophyll							
				H_2O		1% H_2SO_4		5% H_2SO_4		10% H_2SO_4	
Type of mycelium.	Liquid above mesophyll.	Direction of growth.	Length of growth.	Direction of growth.	Length of growth.	Direction of growth.	Length of growth.	Direction of growth.	Length of growth	Direction of growth.	Length of growth.
Germ-tubes	H_2O	Away	5	No change	10	No change	8	Looping	7	Looping	6
Leaf hyphae	H_2O	Away	1	No change	4	No change	4	No change	2	No change	1
Germ-tubes	1% H_2SO_4	Along	1	No change	5	No change	5	No change	3	No change	3
Leaf hyphae	1% H_2SO_4	Away	1	No change	4	No change	3	No change	2	No change	1
Germ-tubes	5% H_2SO_4	Along	1	No change	5	No change	4	No change	2	No change	2
Leaf hyphae	5% H_2SO_4	—	0	Away	4	No change	4	No change	2	No change	1

* Length of growth was estimated by eye on a scale 0–10.

† Looping denotes a change in direction of growth of the germ-tubes or the leaf hyphae.

Discussion

A red coloration in the germ-tubes, appressoria, and substomatal vesicles was found to be associated with attempted and successful nuclear division (Dickinson, II, 1949). This red colour would seem to be due to the presence of reduced carotin. Thus reduced carotin is associated in the germ-tubes, &c., with attempted and successful nuclear division. Nuclear division would imply a demand for oxygen, for much is required at such a time. If the permeability of the hyphal wall was low, the supply of oxygen through it might well be insufficient for nuclear division. Instead this oxygen demand might be satisfied from the carotin pigment (normally present in the oxidized and colourless state). The immediate disappearance of the red colour on the bursting of the germ-tubes would support this suggestion, and the failure of increased oxygen to prevent the appearance of reduced carotin in the germ-tubes could also indicate a low permeability rate in their hyphal walls. A low permeability would be an advantage to the germ-tubes normally growing outside the host, where the relative humidity is liable to vary.

Inside the leaf, the leaf hyphae grow under more even humidity conditions. The greater permeability of their hyphal walls would be beneficial for growth under such humidity conditions. In addition it is reasonable to suggest that nuclear division in the leaf hyphae would require a similar amount of oxygen to that in the germ-tubes. Yet there is no evidence of the occurrence in them of a red coloration when in the host plant, and so of the presence of reduced carotin. As carotin is present in the germ-tubes, it is probable that it would also be present in the leaf hyphae. Yet growth under water, such as caused a red coloration in the germ-tubes, had no such effect on the leaf hyphae. Growth in some dye solutions did produce red-coloured granules, and a red coloration has been seen in leaf hyphae growing on some membranes. A high permeability of the walls of the leaf hyphae would explain the absence of a demand, during nuclear division under normal conditions, on internal sources for oxygen, such as carotin.

The morphological similarity between the thigmotropic responses of the germ-tubes and the leaf hyphae has already been described (Dickinson, II, IV, 1949). The difference in thigmotropic perception between the germ-tubes and the leaf hyphae (Dickinson, IV, 1949) might also be explained on the basis of a difference in the permeability of their cell-walls, for such would probably be associated with an alteration in the sensitivity to contact impressions. The problem of the nature of the thigmotropic stimulus and its perception will be considered in Part VI of this series.

In Part I (Dickinson, 1949) it was suggested that the positive hydrotropic curvature in the germ-tubes was brought about by the deposition of a film of water on the side of the hypha nearer to the water source. This was supported by the observed slower rate of growth in submerged germ-tubes. Such a slowing down of growth might be due to a lack of oxygen, and this would be more likely to occur in a hypha having a low permeability rather than a high

permeability. No hydrotropic curvature has been observed in leaf hyphae, although the adjacent germ-tubes did 'loop'. The lowered relative humidity did, however, reduce the length of hyphae produced, so indicating that the leaf hyphae were not insensitive to alterations of relative humidity.

Thus, it would appear a reasonable hypothesis that the physiological change from the ectophytic to the endophytic stage of the fungus, which has taken place after substomatal vesicle formation, has been in the permeability of the hyphal wall. Whether this change in the permeability, or an alteration in the readily available oxygen, would induce all the differences observed (see Table I) between the germ-tubes and leaf hyphae remains to be investigated.

Summary

A study has been made of the carotin pigment present in the germ-tubes, appressoria, and substomatal vesicles of *Puccinia triticina*. In the germ-tubes this carotin is present in the oxidized condition, while in the spores, appressoria, &c., it is in the reduced condition. The permeability of the germ-tubes, as indicated by range and rate of dye uptake, is less than that of the leaf hyphae. While both germ-tubes and leaf hyphae are affected by relative humidity, only the former change their direction of growth towards a higher relative humidity.

In view of this evidence it is suggested that the physiological change which takes place at the end of the ectophytic stage (i.e. after substomatal vesicle formation) is a change in the permeability of the cell-wall. Whether such a change in cell-wall permeability could be responsible for all the differences observed between the ectophytic and endophytic stages of *Puccinia triticina* remains to be investigated.

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Studies in the Genus *Elaphoglossum*

IV. The Morphological Series in the Genus and their Phylogenetic Interpretation

BY

P. R. BELL

(*Botany Department, University College, London*)

With eight Figures in the Text

ABSTRACT

A detailed comparison of the steles, of the joints, and aerenchyma of the petioles and of the scales of the frond of eighty-seven species of *Elaphoglossum* has revealed morphological series in each of these characters which can be objectively graded. Although there is no reason to believe that these characters are developmentally or genetically linked, certain grades of them occur together much more frequently than would be expected on a basis of chance. It is argued that the species examined form a representative sample of the genus and that there is no evidence to suggest that the associations detected are due to natural selection. A phylogenetic explanation is advanced with which other features of the genus are found to be in conformity.

Other investigations have shown that the extent to which lateral buds develop in rhizomes is related to the length of the internodes and that stomatal frequency is higher in those species whose fronds are scaly than in those in which they are glabrescent.

PART I

[Annals of Botany, N.S., Vol. XIX, No. 74, April, 1955.]

1. Material

SINCE the last communications (Bell, 1951*a* and *b*), additional material of *Elaphoglossum* has been examined from Jamaica, St. Vincent, Ecuador, Peru, West and East Africa, Madagascar, Ceylon, Malaya, New Guinea, and Marion Island.

The additional Jamaican material comprised sixteen species, namely *E. apodum* (Klf.) Schott, *E. crinitum* (L.) Christ, *E. cubense* (Mett.) C. Chr., *E. erinaceum* (Fée) Moore, *E. glabellum* J. Sm., *E. gramineum* (Jenm.) Urban, *E. herminieri* (Bory) Moore, *E. huacsaro* (Ruiz) Christ, *E. hybridum* (Bory) Moore var. *denudatum* Jenm., *E. leptophyllum* (Fée) Moore, *E. maxoni* Underwood, *E. nematorhizon* Maxon, *E. petiolatum* (Sw.) Urban, *E. siliquoides* (Jenm.) C. Chr., *E. simplex* (Swartz) Schott, and *E. spathulatum* (Bory) Moore. According to Morton (1953) two more species have been recorded from the island (excluding *Rhipidopteris*), namely *E. lindeni* (Bory) Moore and *E. decoratum* (Kunze) Moore. Material of these has not been available for detailed study, but *Bell 15* and *82* from Ecuador are probably *E. lindeni* and, so far as can be ascertained from a limited examination of herbarium specimens, there is no reason to suppose that *E. decoratum* would fall outside the morphological account given here.

The Ecuadorean material was collected in the Andean rain and bush forest and from the páramo, representing an altitudinal range of from 800 to 4,200 metres (Bell, 1953). It has proved impossible to name this collection with finality owing to the confusion in the nomenclature of the genus, particularly of the Andean representatives, which are numerous and still little known. Many of the original descriptions of species are based upon amounts of material which, by modern standards, are quite inadequate. Since the differences between species are often of degree and lie in such features as the proportions of the lamina of the frond or its scaliness, the complete absence of any knowledge of the phenotypic variation of these features renders it impossible to be certain of the distinctness of the species based upon them. The revision of the nomenclature of the genus in a satisfactory manner is not yet possible and for this reason the naming of the Ecuadorean collection has not been attempted at this stage. In place of naming, the material has been sorted according to both internal and external characters. Numbers which have proved identical, or very closely related and without definite evidence of distinctness, have been associated together. In this way forty-six numbers or groups of numbers have

been obtained which have been treated as entities of specific rank, each separated from the other by a precise difference or combination of differences. Reference will be made to these species by quoting the collecting number, a practice which avoids the confusion consequent upon the use of provisional and inaccurate nomenclature. A list of the duplicates in the collection is incorporated into Table V.

The discrimination of species in this way has inevitably been influenced by personal judgement, but the treatment has been cautious and uniform so that it is unlikely that any species have been unjustifiably created. On the other hand, some critical species may have been overlooked, but this is unavoidable in the absence of information which would enable constant genotypic variation to be distinguished from that caused purely by the environment. Every gathering made in Ecuador was divided into two portions, one of which was made into herbarium specimens and the other preserved in spirit for morphological investigation. The herbarium specimens have been deposited in the British Museum (Natural History) and when it is eventually possible to name them there will be no doubt to which species the morphological information given here belongs. Some of the Ecuadorean gatherings proved identical with Jamaican species which had already been examined, namely *Bell 494*, *503*, and *802* which are all *E. tectum*, and *Bell 244* and *272* which are both *E. apodum*.

E. procurrens (Mett.) Moore (*Maxon 4334*), a distinctive creeping species, has been examined from Peru. The material was obtained from a herbarium sheet.

The West African material consisted of *E. aubertii* (Desv.) Moore (*Adams 1730*), *E. cinnamomeum* (Bak.) Diels (*Adams 1702*), *E. isabelense* Brause (*Adams 1676*), *E. kuhnii* Hieron. (*Adams 1720*), and *E. welwitschii* (Bak.) C. Chr. (*Adams 1677*), all of these being collected on Cameroons Mountain. *E. clarenceanum* (Bak.) C. Chr. (*Quintas 1379*) has also been examined from West Africa (San Tomé), together with *E. subcinnamomeum* Hieron. (*Schelpe 2548*) from East Africa, *E. spathulatum* (*Milne-Redhead 4334*) from Northern Rhodesia, and *E. phanerophlebium* C. Chr. (*Perrier de la Bathie 12039*) from Madagascar.

Preserved material of *E. callifolium* (Bl.) Moore, *E. melanostictum* (Bl.) Moore, and *E. yunnanense* (Bak.) C. Chr. has been available for study. Herbarium material of *E. brevifolium* Holtt. (*Ridley 15967*), *E. decurrens* (Desv. non Fée) Moore (*Singapore Field No. 25717*), and *E. peninsulare* Holtt. (*Eryl Smith 2021*) has also been examined.

The Ceylon form of *E. spathulatum* (*Beddome* s.n.) has been compared with that from Africa. *E. bolanicum* Ros. (*Clemens 5545*) from New Guinea, *Rand 3710* from Marion Island, and *E. undulatum* (Willd.) Moore from St. Vincent (*Smith & Smith 1136*) complete the investigation.

In all eighty-seven species of the genus (including *E.* (*Hymenodium*) *crinitum* and *Bell 662* (*Rhipidopteris* sp.)) have now been studied in detail and the results are embodied in the following account.

2. Habit of the Rhizome

Creeping, ascendent, and erect forms were present in the recent as in the earlier collection and the same relation was found between the habit of the plant and the symmetry of the mature stele as previously (Bell, 1950). *E. nematorhizon* from Jamaica, *Bell 346* and *662* from Ecuador, and *E. procurrens* from Peru are noteworthy in possessing thin creeping rhizomes, little more than 1 mm. in diameter and with numerous lateral branches borne alternately on each side of the main axis. These branches correspond in position to the

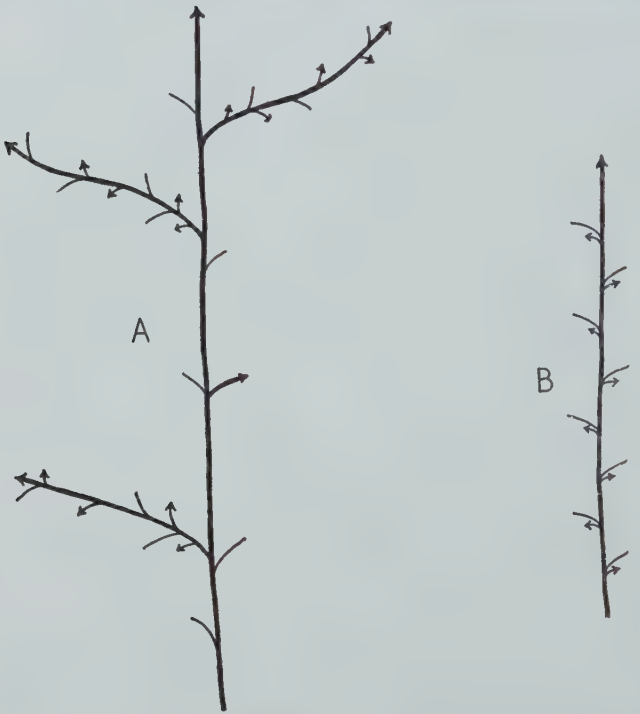

Fig. 1. Diagram to show the growth patterns of the rhizomes of *Bell 346* (A) and *E. latifolium* (B). The latter pattern can be considered a condensed form of the former. Lines terminated by arrow-heads indicate axes, others petioles.

lateral buds occurring in other species of the genus (Fig. 1). This branching character is also present to a less extent in *Bell 100*, *310*, and *665*, where the rhizomes are thicker and of the order of 3 to 5 mm. in diameter.

Bell 97, *742* and the posterior portion of *Bell 765* also occasionally produce long branches. The latter species is peculiar in possessing a rhizome which is procumbent in the posterior portion, but which becomes erect and scandent in the anterior. The long branches are confined to the posterior portion where the petioles are two-ranked.

3. Stelar Structure

The forms of stelar symmetry. To the three forms of stelar symmetry discovered previously a fourth must now be added, intermediate between the

muscosum-form and the *villosum*-form (Bell, 1950; Figs. 4 and 6 respectively). In this new grade bud traces precede all or almost all the leaf traces irrespective of their position, although the stele retains a clear dorsiventrality (Fig. 2). This dorsiventrality is indicated by the fact that it is only from the ventral portion of the stele that root traces are emitted directly, those produced elsewhere always being in association with bud traces. This ventral portion of the

FIG. 2. *Bell 911.* Transverse section of the stele; *v*, ventral meristele; *bt*, bud trace; *bt+r'*, bud trace and associated root trace about to depart from the margin of the ventral meristele; *lts*, strand of the leaf trace; *r*, root trace.

stele occasionally becomes very reduced and there is then a very close approach to radial symmetry.

These four states of stelar symmetry can be arranged in a morphological series of increasing complexity. The dorsiventral two-ranked condition of, for example, *E. latifolium* is the simplest state. This is succeeded by the three- or multi-ranked condition in which the bud traces are found only in association with the ventral rows of leaves and this by the condition similar in all respects, except that bud traces are associated with all or almost all the leaf traces. Lastly comes the radially symmetrical condition. To facilitate reference in the analysis of the results of this investigation these grades of complexity are referred to by consecutive numbering according to the scheme

shown in Table I. The Greek letter α is used to denote the stelar character, and the grade of complexity is indicated by the subscript figure.

It should be noted that the steps in this series are not necessarily phylogenetic steps. The numbering is meant only to indicate a serial relationship, not to suggest that grade 4 has appeared later in the course of evolution than grade 1.

TABLE I

Grades of Stelar Symmetry

	Grade.
Dorsiventral two-ranked condition with bud traces posterior to each leaf trace (e.g. Bell, 1950, Fig. 3)	α_1
Dorsiventral three- or multi-ranked condition with bud traces associated only with the marginal leaf traces (e.g. Bell, 1950, Figs. 4 and 5)	α_2
Dorsiventral three- or multi-ranked condition with bud traces associated with the marginal and most or all of the dorsal leaf traces (e.g. Fig. 2)	α_3
Radially symmetrical condition with bud traces associated with most or all of the leaf traces irrespective of position (e.g. Bell, 1950, Figs. 6 and 7)	α_4

The ontogeny of the stele. This investigation has shown that the mature rhizome of any given species (the maturity of the rhizome being judged by its having reached its maximum diameter and its bearing fronds of the ultimate form and size) always possesses a stele of the same grade of complexity. It has frequently been observed that species possessing α_2 symmetry in the mature rhizome show α_1 symmetry in the juvenile portions. The number of internodes through which α_1 symmetry persists in these species varies considerably in different specimens and may depend upon the amount of nutrition available to the plant. On the other hand, where the mature rhizome shows a stele of α_3 symmetry, this appears always to be rapidly acquired in the development of the sporeling. There is usually a short initial phase of α_1 symmetry and then an immediate progression to α_3. In two instances, however, in species with α_2 symmetry in the mature condition, an exceptional bud trace has been found in association with a leaf trace in the median region (*Bell 511* and *816*). This suggests that the ontogenetic relationship between the second and third grades of stelar symmetry is not very distant.

The peculiar habit of *Bell 765*, a portion of the rhizome of which is shown in Fig. 3, is associated with a succession of stelar conditions at different levels. In the posterior juvenile portion, which is not shown in the diagram, there is regular α_1 symmetry. As the rhizome becomes erect, leaf traces are inserted into the morphological dorsal region and the stele acquires a multi-ranked α_2 symmetry. There persists, as shown in the diagram, a morphologically ventral region from which alone root traces are emitted. In the specimen examined and figured, α_2 symmetry was retained up to the level *A*; above this point root and bud traces ceased to be emitted and the trace to the petiole marked *B* in the diagram arose from the middle of the portion of the stele which had hitherto produced only root traces. Above this point the symmetry

was quite radial. Although in this species there was a complete transition from dorsiventral to radial symmetry, the latter differs from the α_4 symmetry of *E. villosum* and others in the complete absence of root and bud traces. Nevertheless, the existence of this transition does demonstrate that dorsiventrality and radiality are within the morphogenetic compass of one species.

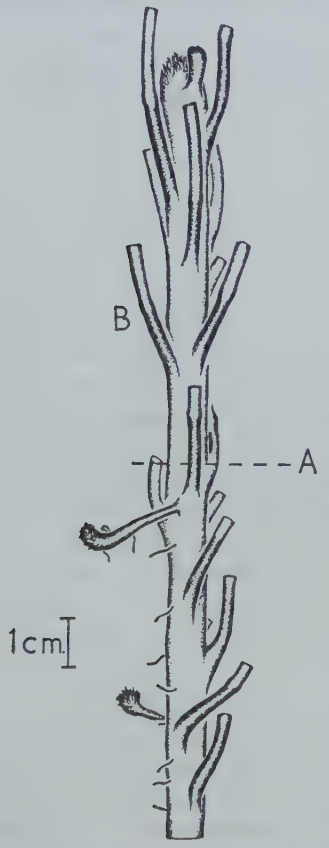

FIG. 3. *Bell 765*. Upper portion of the rhizome. For explanation of A and B see text.

In *Bell 280*, a species with α_4 symmetry, it was found that the mature condition, complete with root and bud traces, was acquired as soon as the stele of the sporeling opened, without intermediate phases of lower order. This species is so similar in habit and general features to *E. villosum* and other species with α_4 symmetry that this rapid acquiring of the mature condition of the stele is probably true of all of them. It contrasts strongly with the protracted transition to radial symmetry which is seen in *Bell 765*.

The relation between the length of the internodes and the development of the lateral bud traces. All the species with prostrate freely branching rhizomes mentioned earlier have steles of α_1 symmetry. Not only do the positions of the branches correspond to the positions of lateral buds in unbranched species,

but also the origin of the vascular supply to the lateral apex bears precisely the same relation to that of the leaf trace on its anterior side in the branched as in the unbranched species. Branching and the production of lateral buds appear, therefore, to be morphologically equivalent, the difference lying in the continued development of the lateral apex in the branched species and its restricted development in the unbranched. There is a striking relation between the degree of development of these lateral apices and the length of the internodes. In the freely branching species the internodes are proportionately long. *Bell 346*, for example, which has a rhizome of only 1 mm. diameter, has internodes from 14 to 20 mm. in length and in *Bell 310*, with a rhizome of about 3 mm. diameter, the internodes reach up to 50 mm. In *E. latifolium*, on the other hand, a species with α_1 symmetry, but dormant buds, and a rhizome which reaches 5 mm. or more in diameter, the internodes are of the order of 10 to 15 mm. Internodes of greater length are rarely found in species with α_1 symmetry in which the buds remain dormant. Species with α_2, α_3, and α_4 symmetry have always been found to have shorter internodes and to produce branches very rarely, usually only from necrotic regions or in specimens in which the main apex has been damaged. This extended investigation has, in fact, clearly supported the view expressed earlier (Bell, 1951*a*) that in the genus as a whole there is a progressive reduction in the development of the lateral bud trace in passing from two-ranked dorsiventral to radial symmetry of the stele, this progression being associated with a reduction in the length of the internodes. *Bell 765* shows this relation in one species. Outgrown branches are found only in the basal portion where there is simple α_1 symmetry and the internodes are of the order of 10 mm. As the internodes become shorter and the stele more complex, so the buds become dormant and progressively more reduced.

The evidence presented in the foregoing points to a developmental relationship between the amount of internodal growth and the activity of the lateral meristems. Although it is possible that the production of leaves in a median as well as in a marginal position may actively inhibit the amount of internodal growth, it is also possible that the shortening of internodes in radially symmetrical and multi-ranked species may simply reflect the more rapid initiation of leaf primordia, relative to extension growth, in these species. This latter view is supported by the fact that in two species with rhizomes of similar diameters, but differing in one having a multi-ranked and the other a two-ranked arrangement of the leaves, the buds being confined to the marginal rows of the former, the distance between the marginal insertions of the multi-ranked is often of the same order as the length of the internodes of the two-ranked species. In lengths of axis, morphologically equivalent as well as metrically equal, there are thus more leaf primordia in the multi-ranked than in the two-ranked species. As was pointed out earlier, the anatomical picture is also of extra leaves being intercalated into the dorsal region of the rhizome and superimposed upon a basic two-ranked pattern (Bell, 1950). The series from freely branching two-ranked to multi-ranked and radially symmetrical

species can, therefore, be regarded as one of increasing frequency of initiation of leaf primordia, relative to extension growth, coupled with an increasing depression of the activity of the lateral meristems, culminating in their becoming reduced and almost obsolete. Since, in freely branching species, the lateral meristems develop in spite of the intactness of the apex, their progressive failure to do so as the frequency of the initiation of leaf primordia increases may be due to the increasing utilization in the apical region of the substances necessary for meristematic activity, resulting in a passive rather than an active inhibition of the lateral meristems. On the other hand, the fact that a lateral meristem in the old part of a multi-ranked rhizome, isolated from the main apex by a necrotic area, may sometimes become active suggests that there may also be some direct inhibition of the laterals by the main apex.

The phylogenetic aspects of these morphological relationships are discussed at a later stage.

The significance of the grades of stelar symmetry. The four grades of stelar symmetry must be regarded as stable states, one of which will be characteristic of the mature rhizome of a given species. That they are related genetically is suggested by the transition which is found from the first to the second grade in the ontogeny of some species, from the first to the third grade in others, and the very close approach to the fourth grade which is made by at least one species with the third grade, together with the other transitions noted. If the variation of the genetic factor or factors governing the form of the stele lies, as the morphological evidence suggests, within a closely connected range, there arises the possibility that the different forms of steles found within the genus may have been fairly recently evolved, either since or during its becoming distinct from related polypodiaceous ferns. The phylogeny of the form of the stele is discussed in a later section.

There is one correction to be made to the earlier work. Additional material has shown that the specimen of *E. chartaceum* from Jamaica examined previously was juvenile. The mature rhizome has an α_2 and not an α_1 stele.

4. The Joint and Aerenchyma of the Petiole

The value of the degree of development of the joint and of the aerenchyma at the base of the petiole as a comparative feature has become apparent from the present studies. Until *E. latifolium* was seen in a living state in Jamaica, it was not realized that the parenchymatous tissue at the base of the petiole was actually more prominent than previously illustrated (Bell, 1950; Fig. 2) and formed two flanges, one on each side of the insertion. *E. chartaceum* possesses similar aerenchyma (Fig. 4, A) and the earlier description (Bell, 1950) is erroneous. In life this flange of tissue is pale green and contrasts markedly with the black base of the petiole. Anatomically it consists of loose parenchymatous tissue with large air-spaces. Stomata are present in the epidermis. Where the aerenchyma takes this form it is limited to these sharply defined flanges and is not continued as ascending ridges or stripes. The fibrous zone within the petiole is broken only at the insertion of the flange.

The association between distinct petiolar joints and aerenchymatous flanges. In the material examined aerenchyma of this form has been associated, with only one exception (*Bell 665*), with a very sharp colour change in the petiole near its base. In all of these species, with the sole exception of *Bell 662*, there has been a short localized swelling at this point. These three features are shown in Fig. 4, A. This region of colour change is referred to as the 'joint'. *E. nematorhizon*, *E. procurrens*, and *Bell 346* also have petioles with distinct joints, but in these species they are unswollen and the basal aerenchymatous flanges are missing.

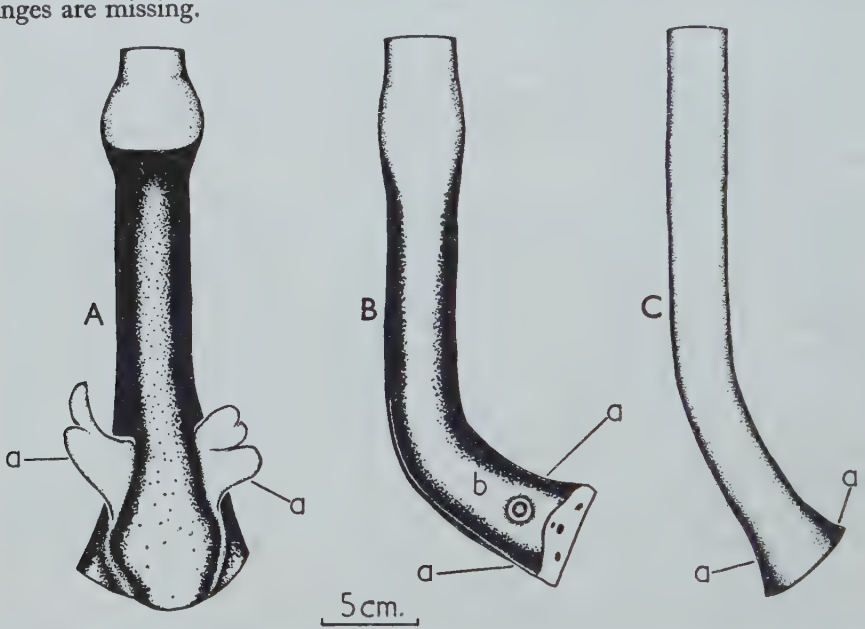

FIG. 4. Bases of the petiole to show the three grades of development of the joint and aerenchyma. A, *E. chartaceum* (β_3 grade); B, *E. muscosum* (β_2 grade); C, *E. villosum* (β_1 grade); *a*, aerenchyma; *b*, bud. For further explanation see text.

The close association which has been found in the genus between the presence of aerenchymatous flanges and a distinct joint in the petiole is unlikely to be an illusion engendered by the sample being unrepresentative or by the fact that the species which have been examined are closely related, since the association occurs in dissimilar species drawn from a wide area. The data are shown in Table II.

That two characters in such close proximity should be developmentally related is not surprising, but the instances of dissociation noted above (*Bell 665*, *E. nematorhizon*, *E. procurrens*, and *Bell 346*) suggest that the two characters are, nevertheless, genetically distinct.

Additional forms of petiole. A large number of species have a petiole similar to that described above, but with both the joint and the limits of the aerenchyma less distinct. The swelling of the joint is less localized and the associated change in colour, although still taking place over a limited length, is

diffused. The aerenchyma forms two low marginal ridges which ascend from the insertion and either die out or continue upwards and become confluent with the margins of the lamina, depending upon the species. The fibrous zone of the petiole is broken by parenchymatous tissue beneath these ridges in some species, but not in others. A petiole of this form is illustrated in Fig. 4, B.

TABLE II

Association of Aerenchymatous Flanges with Distinct Joints in Petioles of Elaphoglossum

Locality.	No. of species with distinct joints (with or without aerenchymatous flanges).	No. of species with aerenchymatous flanges (with or without distinct joints).	No. of species showing both petiolar characters.
Jamaica . .	9	8	8
Ecuador and Peru .	9	8	7
Malaya . . .	5	5	5
West Africa . .	1	1	1

A third and smaller group of species shows no trace of a joint in the petiole at all. The change in colour from the insertion upwards is very slight and takes place gradually over the whole length of the petiole. The aerenchyma forms two very slight ridges at the insertion which ascend for a short distance and then vanish. These features are shown in Fig. 4, C.

TABLE III

Grades of Development of the Petiolar Joint and Aerenchyma

	Grade.
Petiole without joint. Aerenchyma forming marginal ridges, obscure, rarely conspicuous (*Bell 665*), and soon dying out (Fig. 4, C)	β_1
Petiole jointed, but joint not sharply localized and not, or only a little, prominent. Associated colour change diffuse. Aerenchyma forming narrow marginal ridges, sometimes crenulate, but always < 1 mm. high, dying out before the joint or ascending as faint marginal stripes (Fig. 4, B)	β_2
Joint well defined, localized, and usually prominent, associated with a sharp colour change. Aerenchyma in the form of pegs or flanges $\geqslant 1$ mm. high and confined to the base of the petiole near its insertion (Fig. 4, A), rarely absent (*E. nematorhizon*, *E. procurrens*, and *Bell 346*)	β_3

The grading of petiolar development. The three forms of petiole described in the foregoing are obviously related and form a morphological series. The degree of development of the joint and of the aerenchyma of the petiole has proved constant for each species. A system of grading, similar to that used for the steles, is therefore introduced. The symbol β is used to denote the petiolar character, and its grade of development, determined according to the scheme set forth in Table III, is again indicated by the subscript figure. As with the steles, the numbering merely indicates the serial relationship of the grades in

the order of increasing complexity without any phylogenetic connotation. No association is to be assumed between the grades of this series and those of the stelar series.

The close similarity between the forms of the petiole suggests that the genetic factor or factors responsible for their development have much in common. There is again the possibility that this series of forms has arisen in the evolution of the genus. This is discussed at a later stage.

According to Troll (1933), aerenchymatous pegs and flanges on the petiole are of importance to the young developing leaf. This may be so in ferns in which the young leaf is enveloped in a mucilaginous sheath, as were those of the ferns investigated by Troll, but in *Elaphoglossum*, in which there is no mucilage, they seem to have no adaptative significance. The leaves of species without aerenchymatous flanges are no less well formed than those of species in which they occur.

5. The Scales of the Frond

Although several additional forms of scales have been discovered in this extended investigation, they do not fall outside a morphological series of which minute glandular emergences on the one hand, and bristle and peltate scales on the other, are the extreme states. A detailed comparison of the members of this series has led to the view that they are all developmentally related, the differences between the scales of different species being of degree rather than absolute. The evidence for this view is assembled under the following four heads.

i. *The relation between glandular emergences and laminate scales.* In a number of species a whole range of appendages has been found on the mature frond extending from glandular emergences to laminate scales. The range from the lower surface of the lamina of the frond of *E. nematorhizon* is shown in Fig. 5. The simplest structures are single glandular hairs and the most complex laminate scales with basal attachment. The occurrence of numerous intermediate states suggests that only one ontogenetic process is involved, but that the time available for development before the onset of maturity varies from the primordium of one emergence to that of another. In the larger scales growth appears to be initially directed into the formation of a lamina and finally into that of glandular processes, whereas in the smaller scales growth is entirely directed into the formation of a gland or glandular processes. A range of scales quite similar to that on the lower surface of the frond of *E. nematorhizon* is found on both surfaces of the frond of *Bell 346* and *662* (*Rhipidopteris* sp.).

A similar situation exists in *E. chartaceum*. Here, if a newly mature frond is examined, structures ranging from branched glandular hairs, similar to those of *E. pallidum* (Bell, 1951*b*, Fig. 7) to minute laminate scales fringed with glandular processes will be found scattered on both surfaces. Ranges of quite similar structures are found on the fronds of *E. latifolium*, *E. sellowianum*, *E. isabelense*, *Bell 260A*, *264*, *310*, and *742*, and probably in *E. callifolium*, *E.*

decurrens, and *E. melanostictum*, although the material of these last three species was a little too old to show this feature well. An intermediate and an extreme member of one of these ranges would resemble the structures shown in Bell, 1951*b*, Figs. 5 and 6 respectively. Many of these glandular hairs and minute scales are shed from the mature frond and are therefore difficult to find on old specimens.

A range of scales from the lower surface of the lamina of the frond of *Bell 666* is shown in Fig. 6. Here, although the scales are of different form, the

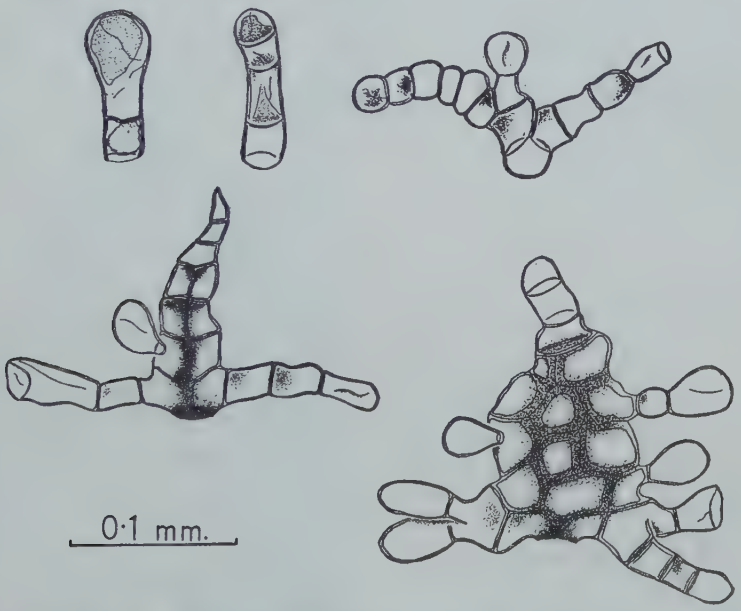

FIG. 5. *E. nematorhizon*. Range of scales from the lower surface of the lamina of the frond. The simple and branched hairs stand away from the surface, but the laminate structures are prostrate.

relationship between the extremes is again one of differential development. The intermediate scales show a progressive unilateral expansion of the lamina. Not only are the extremes connected by this series, but also the fully expanded scale retains on the unexpanded side of the pedicel a fringe of glandular appendages identical to those of the simple emergence.

These ranges of scales described in the foregoing have all occurred on mature fronds and the deep pigmentation and thickened walls of the cells composing them point to the fact that they are fully differentiated. The existence of these ranges is taken as evidence that glandular emergences and simple laminate scales are developmentally related and that the processes which give rise to them are of the same kind, both physiologically and genetically.

ii. *The relation between laminate scales with basal attachment and laminate scales with lobed bases and immersed attachment.* In a large group of species the base of the scale is cordate and the attachment is at the origin of the sinus.

Although in these species the scales of the lamina of the frond are generally of one kind only, transitional scales are found on the petioles of a number of species. An example of these transitional forms is seen in *E. eggersii*. The scales of the lower part of the petiole have a simple basal attachment, but on

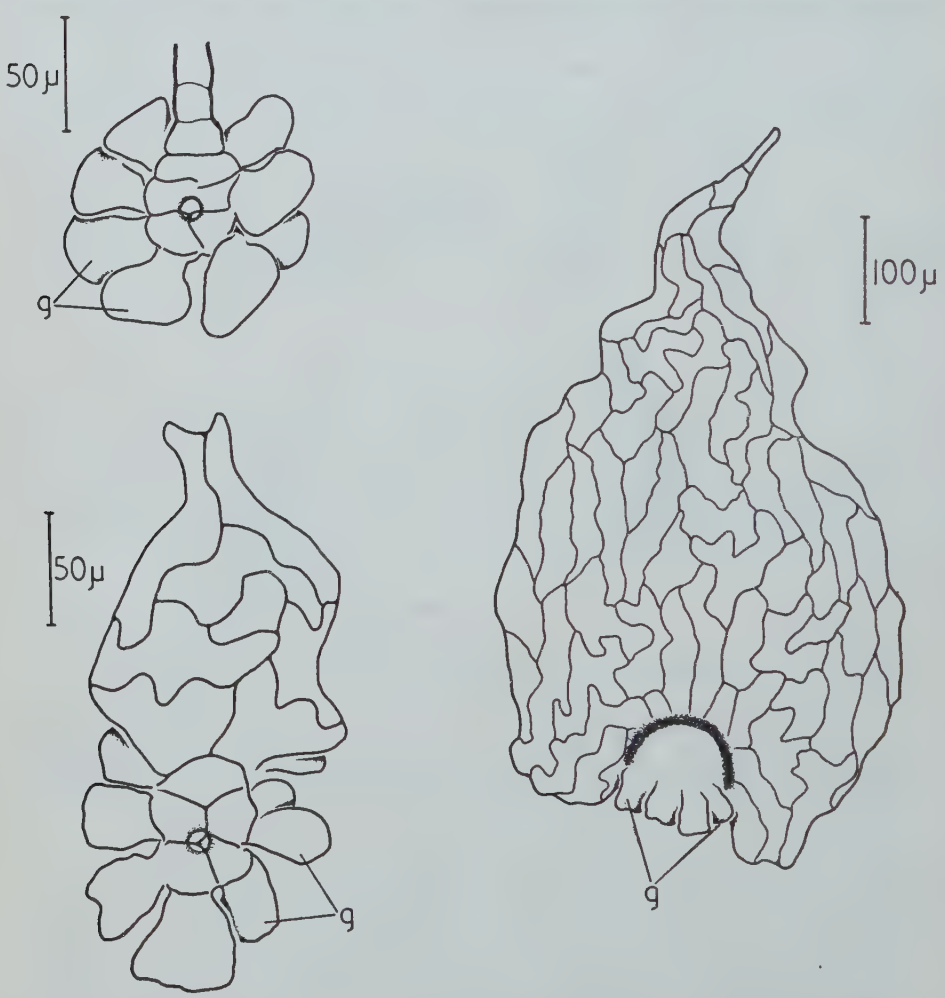

FIG. 6. *Bell 666*. Range of scales from the lower surface of the lamina; *g*, glandular appendage. For further explanation see text.

approaching the lamina the base of the scale gradually becomes cordate until the typical scales of the lamina with deeply cordate bases are reached.

Developmentally the two forms of scale differ in the disposition of the growth of their laminae in relation to the pedicel. The occurrence of transitional forms is again taken as evidence that the two extremes are variants of one ontogenetic process.

iii. *The relation between flat laminate scales and bristle scales.* The bristle

scale (see Bell, 1951*b*, Fig. 8) can be considered as a laminate scale with immersed attachment and with the margins revolute almost to the apex (not involute, as was stated in error in the earlier communication). Although the lobes of the sinus remain adpressed to the surface of the frond, the base of the scale is deeply cochleariform, so that the scale from almost immediately above

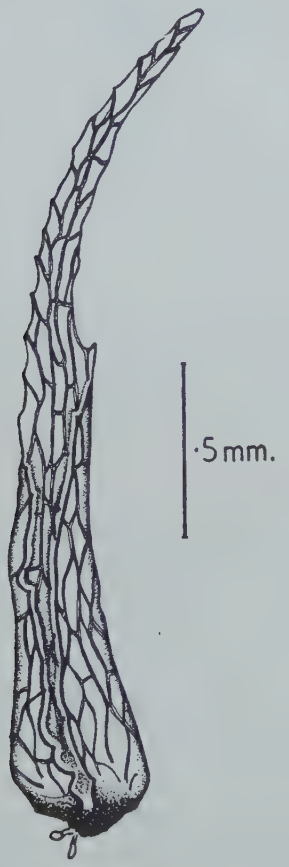

Fig. 7. *E. nematorhizon.* Scale from the upper surface of the lamina of the frond.

the attachment stands at right angles to the surface. Scales intermediate between the flat laminate type and bristle scales have now been found as mature scales on the fronds of a number of species, namely *E. nematorhizon* (Fig. 7), *Bell 81*, *231*, and *487*. Those of *E. nematorhizon* occur only on the upper surface of the frond. The base of these scales is shallowly cochleariform, as a consequence of which the scale stands away from the surface of the frond, but not completely at right angles to it, and the margins of the scales are reflexed below, but not above. A continuation of these tendencies would lead to the bristle scale of *E. villosum* and other species.

In *E. apodum*, *E. crinitum*, *E. cubense*, *E. spathulatum* var. *pusillum*, and *Bell*

568 and *855*, transitional scales occur on the upper part of the petiole, intermediate between the bristle scales of the lamina and the flat scales of the base of the petiole. Again, in *E. aubertii* the frond has fully formed bristle scales at the margin, but more open transitional scales on the upper and lower surfaces.

These facts are taken as evidence of the close ontogenetic relationship of the bristle scale to the flat laminate scale.

TABLE IV

Grades of Development of the Scales of the Lamina of the Frond

	Grade.
Simple glandular hairs Branched glandular hairs Capitate glandular emergences	γ_1
Laminate scales with basal attachment	γ_2
Laminate scales with cordate or lobed bases and the attachment at the base of the sinus Laminate scales standing away from the surface, the base tending to be cochleariform, but the margins revolute in part only	γ_3
Bristle scales (as described in text) Peltate scales	γ_4

iv. *The relation between flat laminate scales and peltate scales.* Radially symmetrical peltate scales occur in *E. tectum* and *Bell 274*. In *E. tectum* a mixture of scales is found on the upper part of the petiole, some of them peltate, others with sinuses and occasional intermediate scales with the base lobed, but the sinus not penetrating to the attachment. In *Bell 274* peltate scales occur on the upper surface of the frond, but near the margin is a fringe of basally attached scales. Between the two forms there are occasional intermediate scales which are asymmetrically peltate and with a shallow lobing at the base which does not penetrate to the attachment.

These transitions are regarded as indicating a close ontogenetic relationship between the peltate scale and the flat laminate scale.

The evidence presented in the foregoing, taken as a whole, points to a morphological and genetic continuity running through all the forms of scales occurring on the fronds of species of *Elaphoglossum*. No herbarium specimens have been seen nor are there any scales described by Christ (1900) which would contradict this view.

It is interesting to note that Troll (1933) records all intermediates between mucilaginous hairs, both simple and branched, and fully formed laminate scales on the rachis of *Dryopteris sumatrana*.

The grading of the morphological series. It has been found that the form or range of form of the scales on the frond is constant for a given species, which justifies the great diagnostic value which has been placed upon them in the taxonomy of the genus. The series can therefore be graded, with the know-

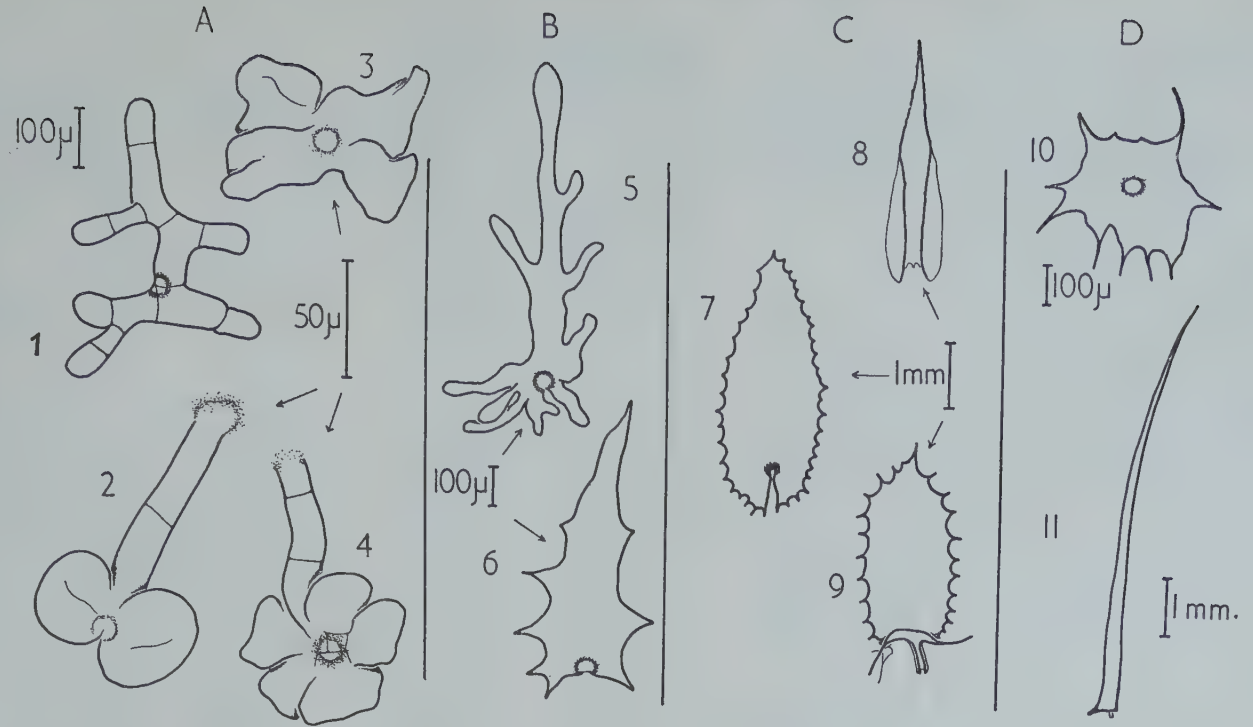

FIG. 8. Diagram showing the principal forms of the scales of the lamina of the frond in *Elaphoglossum* and their attachments. Section A (γ_1): *1*, branched glandular hair from the upper surface of *E. glabellum*; *2*, glandular emergence from the upper surface of *Bell 434*; *3*, squamule from the upper surface of *Bell 266*; *4*, glandular squamule from upper surface of *E. gramineum*. Section B (γ_2): *5*, small laminate scale with glandular processes from the upper surface of *E. chartaceum*; *6*, small laminate scale from the upper surface of *E. petiolatum*. Section C (γ_3): *7*, scale from the upper surface of *Bell 100*; *8*, scale from the upper surface of *Bell 81*; *9*, scale from the lower surface of *Bell 701*. Section D (γ_4): *10*, peltate scale from upper surface of *E. tectum*; *11*, bristle scale from the lower surface of *Bell 280*. The accompanying measurements are intended to give only an indication of the order of size of the various forms of appendage.

ledge that each species will possess its characteristic grade of scales, as with the other characters which have been considered.

The symbol γ is used to denote that the grades refer to the scales of the frond. Four grades are recognized and defined as shown in Table IV. In addition, the principal forms of scales occurring in the genus are shown in Fig. 8, where they are grouped according to the grading. It should be noted that the drawings are of different magnifications in this figure. The structures represented on the left are microscopic, those on the right are macroscopic.

In connexion with the grading, the following two points should be noted:

(*a*) In the second grade, glandular processes directed downwards on each side of the attachment, as in the scale of *E. chartaceum* (Fig. 8, 5), are not regarded as auricles forming a sinus. The lobes which border a sinus are always of similar areolation to the main lamina of the scale and are more than one cell broad at the base.

(*b*) Peltate scales and bristle scales are placed together in the fourth grade since the evidence presented above points to the two forms being related to the flat laminate scale in an equivalent way. Each shows a morphological progression from the flat laminate scale and they can be related only by way of such a scale.

Although the classification and grading of the scales has not been so immediately evident as that of the steles and petiolar characters, the morphological series shown by them is similar to those described for the two previous characters. The remarks made there concerning the numbering of the grades and their possible, but not necessary, phylogenetic significance apply here as well.

The relation between the grade of the scales and their density. Scales of the first and second grades are always scattered on the frond. Those of the third grade are often densely packed and imbricate, sometimes forming on the lower surface of the lamina a thick continuous felt. Scales of the fourth grade are usually distantly scattered.

6. The Results of the Comparative Examination

Using the symbols defined in the foregoing, the results of this comparative examination of the genus *Elaphoglossum* can be expressed in tabular form (Table V).

In this table the grade of the stele accorded to a species is determined by its form in mature plants. That of the scales of the frond indicates, where there is a range of these structures, as in *E. nematorhizon* and other species, the highest grade of the range. In this connexion it should be mentioned that simple glandular hairs (one of the categories of grade 1) have never been found alone on a frond, but always in association with a higher member of the morphological series. Some simple glandular hairs are present on the young fronds of all species, but they persist in conspicuous quantity in only a few

TABLE V

The Analysis of 87 Species of Elaphoglossum in Terms of the Characters α, β, and γ

Species.	Grading. α.	β.	γ.
Jamaica			
E. apodum	2	2	4
chartaceum	2	3	2
crinitum	2	1	4
**cubense*	2	2	4
eggersii	1	2	3
**erinaceum*	2	2	4
glabellum	1	3	2
gramineum	2	2	3
herminieri	2	3	2
hirtum	2	2	3
huacsaro	2	2	2
**hybridum* var. *denudatum*	2	2	2
latifolium	1	3	2
**leptophyllum*	1	3	2
**maxoni*	1	3	2
muscosum	2	2	3
**nematorhizon*	1	3	3
pallidum	2	2	1
**petiolatum*	2	2	2
sellowianum	1	3	2
**siliquoides*	4	1	4
simplex	1	3	2
**spathulatum* var. *pusillum*	4	1	4
tectum	1	2	4
villosum	4	1	4
St. Vincent, Lesser Antilles			
**undulatum*	3	1	3
Ecuador			
(The numbers in parentheses are those of duplicate collections)			
Bell 9 (6, 7, 32, 364, 504, 750)	2	2	3
15 (82)	3	2	4
81	2	1	3
91 (698)	1	2	3
93 (88, 94, 95, 96, 105, 209, 348, 688)	1	2	3
97 (98, 683)	1	1	3
100 (701)	1	2	3
231 (245, 257, 270)	3	1	3
244 (272) (*E. apodum*)	2	2	4
260*A*	1	3	2
264	1	3	2
266	1	2	1
274 (267, 278)	3	2	4
280	4	1	4
281	2	2	3
282	3	2	3
283 (203)	1	2	2
308	2	2	3
309 (212, 463, 520, 663, 767)	2	2	3
310 (796)	1	3	2
346 (350)	1	3	2
434 (528)	2	2	1
441	3	2	1
487 (551, 825)	2	1	3
503 (494, 802) (*E. tectum*)	1	2	4
568	3	2	4
Ecuador—*continued*			
662	1	3	2
665	1	1	2
666	2	2	2
668 (452)	2	2	3
680 (702)	2	2	3
692 (29, 92, 210, 509, 697, 751)	1	3	2
742 (766)	1	3	2
748	2	2	3
749	1	2	3
765	2	2	3
769	1	1	3
798	2	2	1
799	2	2	3
816	2	2	3
820	2	2	1
829 (511)	2	2	2
855	2	2	4
909	2	3	2
910 (912)	2	1	2
911	3	2	2
**E. spathulatum*	2	1	4
Peru			
**procurrens*	1	3	4
West Africa			
aubertii	2	2	4
cinnamomeum	1	1	3
isabelense	1	3	2
kuhnii	3	2	3
welwitschii	2	2	2
San Tomé			
**clarenceanum*	3	1	4
East Africa			
**subcinnamomeum*	1	2	3
Madagascar			
**phanerophlebium*	3	1	4
Central Africa			
**spathulatum*	2	1	4
Ceylon			
**spathulatum*	2	2	4
Malaya			
**brevifolium*	1	3	2
**callifolium*	1	3	2
**decurrens*	1	3	2
melanostictum	1	3	2
peninsulare	1	3	2
**yunnanense*	1	2	3
New Guinea			
**bolanicum*	1	3	2
Marion Island			
**Rand 3710*	1	2	3

* Specimen obtained from herbarium material.

species, notably, amongst those examined, on the fronds of *E. spathulatum* var. *pusillum*, *E. siliquoides*, *E. villosum*, and *Bell 280*, on the fronds of all of which bristle scales occur as well.

7. Other Morphological Features

Scales of the rhizome. These show considerable variation within the genus, but since the variations are in the extent of pigmentation, proportions of the scale, and the form and frequency of marginal glandular processes, it is difficult to define them and to study these scales in a comparative fashion.

E. gramineum and *Bell 820* are noteworthy in possessing only glandular emergencies, quite similar to those of the frond, on the rhizome. Some species possess extremely long ($\geqslant$ 1 cm.) and narrow ($\leqslant$ 1 mm. wide) scales, with very pale pigmentation, which form a shaggy tuft at the apex of the rhizome. The margins are usually weakly repand with simple glands at the cusps. The apex is irregular and glandular. Scales of this form have been found in *E. apodum*, *E. crinitum*, *E. erinaceum*, *E. herminieri*, *E. pallidum*, and in *Bell 568* and *855* (in which they frequently exceed 2 cm. in length). Reference to Table V will show that five of these seven species possess scales of the fourth grade on the fronds.

There are all intermediates connecting this form of scale with ovate-lanceolate and lanceolate forms with basal and immersed attachments, these latter being by far the most common in the genus. The actual pedicel of the scale is occasionally set in slightly from the base or origin of the sinus, but its attachment could hardly be described as peltate, as is done by Holttum (1946). The base of the scales of the rhizome of the Malayan *E. melanostictum* is very complex. The attachment is immersed in a sinus, but the margins of the sinus are fringed with glandular processes and overlap widely. Sometimes one margin is bent back on to the top of the scale and encircles the attachment again, giving the appearance of a small scale lying in the sinus of a larger. In all those examined, however, it has been possible to separate the margins with needles under the microscope and to expose the sinus. There has been no evidence of organic union on the posterior side of the attachment. The rhizome scales of *Bell 692* are sometimes similar at the base.

Hydathodes. These have been found in a few species, their position and structure being quite similar to that described for *E. villosum* (Bell, 1951*b*). They occur in the fronds of *E. aubertii*, *E. cinnamomeum*, *E. nematorhizon*, *E. phanerophlebium*, *E. siliquoides*, *E. spathulatum* var. *pusillum*, *E. undulatum*, *E. villosum*, and *Bell 15*, *81*, *98*, *231*, *280*, *910*, and *911*. Reference to Table V will show that amongst these species there is a preponderance of those possessing γ_3 and γ_4 scales. In at least two species the hydathodes appear to be inconstant. In *E. apodum*, for example, the hydathodes were found in the leaves of the sporeling (of the Jamaican plant), but not in those of the adult. Again, although hydathodes were present in *Bell 98*, they were missing from *Bell 97* and *683* which resembled *98* in all other respects. There does seem

to be a tendency for hydathodes to occur in association with thin membranous fronds; all the above have such fronds, with the exception of *Bell 98*. Other than in this rather limited way, the presence or absence of hydathodes is not a character of comparative value, although, as will be considered later, the presence of hydathodes may be a feature of phylogenetic significance.

The relation between the scaliness of the frond and the habitat. The degree of scaliness of the fronds of a given species, provided they are of the same age, shows little variation, but between species there are considerable differences. Although it may be true, as stated by Christ (1900) and quoted by the present author (Bell, 1950), that the species of high altitudes tend to have densely scaly and those of lower altitudes glabrous mature fronds, species with glabrous or glabrescent fronds and others with densely scaly fronds have been seen growing together in the field at various altitudes. Two pairs of species whose members differed markedly in the scaliness of the lamina, but which occurred either together or not very distant from each other, were particularly noted; namely *E. sellowianum* and *E. eggersii* from Jamaica and *Bell 267* and *281* from Ecuador.

E. sellowianum and *E. eggersii* were both seen growing on banks by trails in apparently quite similar environmental conditions. Both species occurred in more or less the same range of altitude. The fronds of *E. sellowianum* are of similar proportions to those of *E. eggersii*, though larger, but the former are glabrescent and have only scattered minute scales, while the latter, particularly on the lower surface of the lamina, have a dense covering of imbricate scales tangled together by their ciliate processes. *E. eggersii*, according to what is commonly believed to be the function of scales, would seem to be particularly well adapted to resist desiccation. It is true that *E. eggersii* was found growing on loam and *E. sellowianum* on the bases of trunks and decaying vegetable matter, but in all the situations in which they were seen *E. eggersii* seemed no more likely to suffer drying out than *E. sellowianum*.

The Ecuadorean pair, *Bell 267* and *281*, were growing together in banks in the rain forest around the confluence of the Toachi and Pilatón rivers, about 50 km. west of Quito. Both were growing upon peaty material amongst a dense mat of vegetation on rocky ledges. At this altitude, between 500 and 1,000 metres, there was almost continuous rainfall, except for occasional clear mornings. Even then the sky would be clouded by mid-morning and by midday the rain would be falling steadily. During these spells of early morning sun the steaming of the earth and vegetation must have maintained the humidity near, if not at, saturation. There are no meteorological data for this region to show whether these conditions are regularly maintained throughout the year, but the observations made by A. Schultze-Rhonhof (quoted by Diels (1937)) for one year (1935–6) at San Carlos de los Colorados indicate very little change in temperature (which remained within the range 22–24° C.) or wetness during this time. San Carlos de los Colorados does not appear on the most recent map of Ecuador (Sampedro-V., 1950), but according to the map compiled by Wolf (1892) and included in Diels (1937) this settlement is by the

Toachi river, a little farther west than the collecting site. The altitude given by Schultze-Rhonhof is 150 m. above sea-level, but according to the recent map this is clearly too low and it more probably lies between 500 and 1,000 m.

The mature fronds of *Bell 281* show some variations in proportions, but they are always rather longer and narrower than those of *267*. Those of *281* also have a thin coat of closely overlapping and tangled scales beneath, while those of *267* are glabrescent with only distinctly scattered glandular emergences. Both *267* and *281* appeared to be growing equally well. If *281* enjoyed any advantage in possessing a feature possibly enabling it to resist desiccation more effectively, it was not reflected in any greater vigour of growth or frequency of representation than that of *267*. The general lushness of the vegetation and the little information that is available of the climate of the collecting site suggest that desiccation, if it occurs at all, must be very rare.

These observations made in Jamaica and Ecuador indicate that the relationship between the scaliness of the frond in *Elaphoglossum* and the habitat is not one of simple adaptational response, a view which is supported by an examination of the stomatal frequencies of these species.

Stomatal frequency. The stomata are confined to the lower surface of the lamina and their frequency is very variable, reaching almost to 200 per sq. mm. in *E. eggersii*, but not rising above 12 per sq. mm. in *E. villosum*. The latter species has extremely thin papery fronds and is confined to areas of high humidity. Amongst the Ecuadorean species, *Bell 81*, *231*, *434*, *441*, *909*, and *910* all had stomatal frequencies of less than 20 per sq. mm. Of these *Bell 81*, *434*, *909*, and *910* had thin papery fronds, while those of *Bell 231* and *441*, though not papery, were thinner than those of most species. While all species with thin papery fronds had these low stomatal frequencies, equally low frequencies were also found in *E. chartaceum* and *E. crinitum*, both of which have firm fronds of normal thickness.

There is some evidence of a relationship between the density of the scales on the lower surface of the frond and the frequency of the stomata. Amongst the Jamaican species only *E. eggersii*, with its very dense scales, has a stomatal frequency greater than 100 per sq. mm. Amongst the Ecuadorean species, *Bell 282*, *283*, *308*, *309*, *666*, *668*, *680*, *748*, *749*, *799*, and *816* all have stomatal frequencies equal to or greater than 100 per sq. mm. Of these, *Bell 680*, *748*, *749*, *799*, and *816* have the lower surfaces of the fronds completely covered with tightly overlapping scales. In *Bell 282*, *308*, *309*, and *666* the scales are not so dense; the cilia of adjacent scales are tangled together, but the surface of the lamina is not entirely obscured. In *Bell 668* the scales are again close, but the lamina is clearly visible between them. *Bell 283* is exceptional in having a high stomatal frequency, yet a lower surface of the lamina bearing only distantly scattered minute ciliate scales. No species was encountered which had densely scaly fronds and a stomatal frequency of less than 100 per sq. mm., nor any glabrescent species (except *283*) with a high stomatal frequency. In most species the stomatal frequency falls between 20 and 100 per

sq. mm. and the scales of the lower surface of the lamina are either clearly separated or absent.

It is difficult to know the full significance of these results, since stomatal frequency may, as was shown by Salisbury (1927–8) in the Angiosperms, be related to the environment in which the leaf develops, but there seems to be a tendency for the densely scaly fronds to have higher stomatal frequencies. The two pairs of species discussed earlier (p. 193) provide an opportunity to test the relationship between scaliness and stomatal frequency. As the members of each of these pairs enjoyed the same environment and appeared to be growing equally well, it follows that any clear differences in stomatal frequencies between them may be related to the differences in scaliness of the lower surfaces of the fronds. If the relationship is the same in each pair, then this, together with the general observations already made, is strong evidence that frequency of scales and frequency of stomata are not independent of each other.

In order to compare these frequencies it was first necessary to determine whether within the plants themselves there was any constancy in this feature, since in the Angiosperms the stomatal frequency is so variable (Salisbury, 1927–8) that it would be difficult, if not impossible, to make comparisons between species. Accordingly five fronds were taken of each species and twenty estimates of stomatal frequency were made on each. Only mature fronds were chosen, and the counts were made in the central region of the lamina, approximately midway between the midrib and the margin. Since there is a slight change in the epidermal cells immediately beneath the lateral veins, counts were made only between the veins. The counting was done by means of a square grid inserted into the eyepiece of the microscope, the stomata lying within the square being counted and also those cut by the top or left-hand margins or included in the top right-hand angle. Those cut by the other two margins or included in the bottom left-hand angle were ignored. In *Elaphoglossum* the stomata are aligned with their long axes parallel to the lateral veins and the wall of the mother cell joins those of the guard cells on the marginal side of the stoma (Bell, 1951*b*, Fig. 3, A and B; the margin of the lamina is parallel to the top of the drawing). Consequently, in order to obtain strictly comparable counts, the long axes of the stomata were arranged parallel to the top of the square and the insertions of the guard cells in the mother cells to the left in every case.

The results of this investigation are shown in Tables VI and VII. The extent of the variation between fronds can be tested by an analysis of the variance as shown in Table VI. It is seen that in all species, with the exception of *Bell 281*, the stomatal frequency in the central region of the mature fronds of the plants examined can be regarded, for practical purposes, as constant, since the variation between fronds is not significantly greater than would be expected from errors of sampling. If more than approximately 7 per cent. of the variance was between fronds it would be detected by the significance test. In *Bell 281* the variation between fronds is larger than could justifiably

be attributed to sampling errors, but, even so, the variance between fronds is still less than one-fifth of the whole and the means for each frond fall within quite a narrow range. The fronds of this species showed some variation in proportions which may well be reflected in the stomatal counts.

TABLE VI

The Variation in the Number of Stomata per Unit Area in Species of Elaphoglossum

(*a*) *E. sellowianum*

(3 mature fronds taken from one plant, 2 from another)

Analysis of variance.	Degrees of freedom.	Sum of squares.	Mean square.
Within fronds	95	79·35	0·836
Between fronds	4	7·40	1·850

Ratio of mean squares 2·21 $P > 0.05$
Mean of all measurements 3·45 S.E. 0·14;

(*b*) *E. eggersii*

(5 mature fronds taken from one plant)

Analysis of variance.	Degrees of freedom.	Sum of squares.	Mean square.
Within fronds	95	229·90	2·420
Between fronds	4	12·06	3·015

Ratio of mean squares 1·37 $P > 0.02$
Mean of all measurements 9·02 S.E. 0·17;

(*c*) *Bell 267*

(5 mature fronds taken from one plant)

Analysis of variance.	Degrees of freedom.	Sum of squares.	Mean square.
Within fronds	95	56·80	0·579
Between fronds	4	5·16	1·290

Ratio of mean squares 2·23 $P > 0.05$
Mean of all measurements 1·9 S.E. 0·11;

(*d*) *Bell 281*

(3 mature fronds taken from one plant, 2 from another)

Analysis of variance.	Degrees of freedom.	Sum of squares.	Mean square.
Within fronds	95	70·25	0·739
Between fronds	4	13·86	3·465

Ratio of mean squares 4·68 $0.001 < P < 0.01$
Means of individual fronds 3·75, 3·95, 4·10, 4·20, and 4·85
Mean of all measurements 4·17 S.E. 0·19.

Although only one plant of *E. eggersii* and of *Bell 267* was available for investigation, the results obtained with *E. sellowianum* and *Bell 281*, in both of which fronds were taken from two plants, indicate that the stomatal frequency estimated in the manner described is either constant for a given species or varies within a narrow range. Having demonstrated this it is now possible to compare the stomatal frequencies of the members of each pair of species. The

result of this comparison is set out in Table VII. It is clear that the stomatal frequencies of the species with densely scaly fronds are substantially higher than those of the species with glabrescent fronds.

There are two possible explanations of this fact, dismissing as unlikely, in view of the general observations made upon the whole Ecuadorean collection, the possibility of its being fortuitous. In the first place it may be that the thick investment of scales is such an impediment to gaseous exchange through the stomata that, as a condition of survival of the species in its evolution, a genetic tendency towards increasing density of the scales has necessarily been accom-

TABLE VII

Stomatal Frequency, Stomatal Index, Mean Dimensions of Stomata, and Mean Area of Epidermal Cells in Elaphoglossum

	Stomatal frequency (see note 1 below).	Stomatal index.	Mean dimensions of stomata (see note 2 below). Length.	Breadth.	Estimated mean area of epidermal cells (in sq. mm. $\times 10^{-3}$).
E. sellowianum	3·45 S.E. 0·14	19·89 S.E. 0·86	13·92 S.E. 0·09	10·97 S.E. 0·12	2·80
E. eggersii .	9·02 S.E. 0·17	25·98 S.E. 0·59	9·81 S.E. 0·17	8·77 S.E. 0·07	1·54
Bell 267 .	1·90 S.E. 0·11	20·42 S.E. 0·92	11·88 S.E. 0·10	9·12 S.E. 0·12	4·30
Bell 281 .	4·17 S.E. 0·19	23·92 S.E. 0·83	9·34 S.E. 0·09	8·84 S.E. 0·15	3·34

Note 1. Stomatal frequency per sq. mm. can be obtained from these figures by dividing them by 0·04162, the area in sq. mm. of the image of the grid superimposed upon the field.

Note 2. Stomatal size in μ can by obtained by multiplying these dimensions by the factor 3·77.

panied by a tendency towards increasing frequency of the stomata. On the other hand, it may be that the metabolic demands of the developing scales are such that fewer metabolites are available for the development and expansion of the epidermis, so that more stomata are included in the unit area. Evidence in support of this view is given by the facts that where the stomata are very numerous they are also smaller (Table VII) and that the average size of the epidermal cells diminishes with increasing frequency of the stomata.

It will be noted, too, that the *stomatal index* is different for each species, being higher where the stomatal frequency is higher. If, in densely scaly species, the developing epidermis does indeed suffer relative starvation, as suggested above, then the rise in stomatal index could be explained by the epidermal initials failing to divide as frequently in the scaly as in the glabrescent species, the stomatal initials being little or no less numerous in the former than in the latter. It is well known that starvation alters the proportions of tissues in plants, some tissues appearing to have a prior claim on the available metabolites. If the densely scaly leaf has evolved from the less scaly, the

stomata and the epidermal cells may similarly be unequally affected by the increasing diversion of nutrients to the developing scales, the growth and multiplication of the epidermal cells suffering a much greater restriction than the differentiation of the stomata.

Although in these observations different species have been compared, the genus is so uniform that they are probably not distant genetically and the development of the leaves in all species has much in common. The evidence presented here indicates that the size of the stomata and their frequency are not independent and that they are both influenced by the number and size of the appendages produced by the epidermis. That the epidermis suffers relative starvation where these appendages are large and numerous seems a possible explanation of the smaller stomata and epidermal cells found in these conditions. It is interesting to note that Salisbury (1927–8), investigating a large number of British plants, came to the conclusion that variations in stomatal index within a species were due to internal nutritional factors and were not adaptative in any way.

The relation of the stomata to the epidermal cells. Although the stomata of different species frequently differ in mean size and there is a tendency for the smaller stomata to be more nearly circular in outline, there are no marked variations in the form of the stomata which assist in the comparative study of the genus. The differences in the position of the stomata in relation to the epidermal cells brought out earlier (Bell, 1951*b*) have been confirmed by the later investigations. In species with low grades in the α, β, and γ series there is a tendency for the stoma to be surrounded by two or more epidermal cells (as in Bell, 1951*b*, Fig. 3, A) and in those with high grades in these series the tendency is for each stoma to lie within an epidermal cell (as in Bell, 1951*b*, Fig. 3, B). It is impossible to grade this feature and use it in a comparative way, but it may have phylogenetic implications which are considered later.

Lignification of the xylem of the root trace. As recorded earlier (Bell, 1951*a*), the central metaxylem tracheides of the root trace are sometimes unlignified. This feature has frequently been observed, but never in species in which low grades in the α, β, and γ series are associated together. It may occur in these species, but, if so, it must be much rarer than in others.

The fertile frond. The fertile fronds frequently have proportions different from those of the sterile. They are not produced very abundantly and those of the twenty-four species which have been examined have not yielded any information of comparative value. Although the fertile surface appears macroscopically to be uniform and continuous, by cutting the lamina transversely to the lateral veins it has been found in twenty species that there is a clear aggregation of the insertions of the sporangial stalks beneath these veins. In a young fertile frond the sporangial stalks are longer and the sporangia more mature in this region, indicating that the initiation of the sporangia has begun there. Subsequently sporangia arise between the veins and the sorus becomes mixed in age, but the precocity of the portion of the epidermis immediately beneath the veins remains reflected in the greater density of sporangia in this

part. A precisely similar arrangement of the sporangia has been reported in a number of other Acrostichoid ferns (Schumann, 1915). In all the species examined the number of spores in the sporangium has equalled or approached 64 and imperfect spores have occurred only rarely. The number of cells in the annulus of the sporangium usually varies between 10 and 12, although occasionally sporangia with as many as 14 cells in the annulus have been seen. The stalk of the sporangium consists of two ranks of cells below, but three above, and frequently bears a simple gland just beneath the sporangium.

The spores are enclosed in a perispore. The naked spores are bilaterally symmetrical and show some variation in size according to the species. The spores of the fertile species in the collections have fallen within the size limits recorded by Selling (1946) for the spores of a number of Hawaiian species.

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(Part II to follow)

Periodicity of Spore Discharge in *Daldinia*

BY
C. T. INGOLD
AND
V. J. COX
(*Birkbeck College, University of London*)

With five Figures in the Text

Abstract

In *Daldinia concentrica* spore discharge under natural conditions is periodic, most spores being discharged during the night and few in the daytime. An experimental study has been made of discharge under controlled conditions of light and temperature. In continuous darkness periodic discharge was maintained for 12 days, but then, although spore output continued, it ceased to be periodic. Return to alternating light (12 hrs., 100 f.c.) and darkness (12 hrs.) at once re-established the periodicity. In continuous light (100 f.c.) periodic discharge ceased after 2 to 3 days, but was immediately re-established in alternating light (12 hrs.) and darkness (12 hrs.). When the fungus was placed under conditions of alternating light and darkness each of 6 hours' duration, two peaks of spore-output were soon developed in the 24-hour period. The experiments suggest that the natural periodicity is determined by the alternation of day and night.

Introduction

IN *Daldinia concentrica*, as in most Pyrenomycetes that have been tested, violent discharge of ascospores is periodic. In *Daldinia* the maximum rate of discharge is at night, and during the daytime few spores are shed. Since this nocturnal discharge was originally reported (Ingold, 1946) it has been repeatedly verified using many different stromata. The present work was undertaken to investigate the periodicity and in particular to try to decide if it is induced by the alternation of darkness and light.

Before describing the experimental work certain additional facts about spore discharge in *Daldinia* need to be briefly stated. Firstly, the perithecial stroma, which lasts for one season only, commences to discharge its ascospores about the middle of May and continues to do so nightly until some time in September. A detached stroma brought into the laboratory liberates its spores for a few weeks, and during that time the perithecia obtain the water necessary for discharge from the reserve in the stromatal tissue. At the beginning of its spore-discharge period a stroma has a density of just over 1·0, but by the time discharge ceases this is reduced to about 0·3. As drying occurs there is virtually no change in the volume of the stroma, due largely to its extremely hard rind. In its water relations *Daldinia* is unusual amongst Pyrenomycetes. Most

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members of this group can continue to discharge their ascospores only when supplied with an external source of water. Ascospores in *Daldinia* are normally violently discharged to a distance of about 1·5 cm. Sometimes, however, instead of being shot away they escape from the perithecium as a spore-tendril. This occurs particularly towards the end of the active life of a stroma.

Method

The apparatus used in studying the daily march of spore discharge is illustrated in Fig. 1. The stroma (A) was placed on a model-railway truck (B) and covered by a casing (C) having a transparent top provided with a slit approximately 1 cm. wide. The truck ran on a short length of model-railway

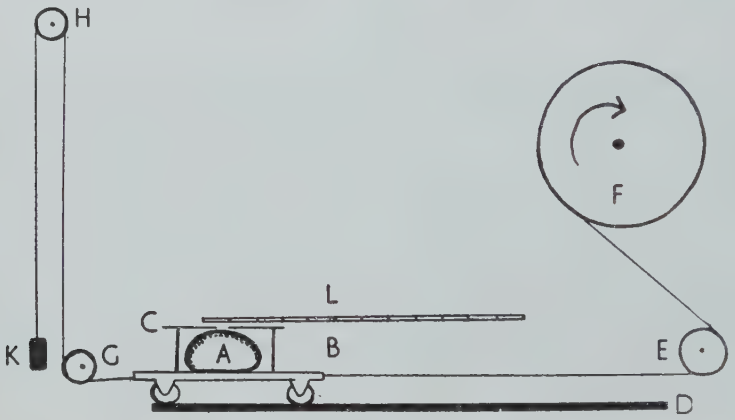


Fig. 1. Diagram of apparatus for studying the rate of spore discharge in *Daldinia*. For explanation see text.

track (D), being drawn along at a uniform rate. A thread from the front of the truck after passing round a pulley (E) was attached to the circumference of a larger pulley-wheel (F) screwed to the axis of a clock making one rotation in 24 hours. This gave the truck a speed of 0·86 cm./hr. A thread at the rear of the truck passed by way of two pulleys (G and H) to a weight (K). This merely acted as a mechanism for steadying the truck and for keeping the leading thread taut. The sticky spores, discharged from the area of the stroma immediately below the slit in the casing, were caught on twelve glass slides (L). These were $7 \times 1{\cdot}72$ cm., the width of each corresponding with the distance travelled by the truck in 2 hours. The slides were kept in position by resting on a framework (not shown in Fig. 1) consisting essentially of two long parallel horizontal bars held at the appropriate height by metal legs. It was so arranged that the interval between the upper surface of the stroma and the under surface of the slides was about 0·5 cm. At the start of a day's run the truck was so adjusted that the slit above the stroma was below the slide on the extreme left, thus each slide received the spores discharged by the exposed portion of the stroma over a period of 2 hours.

The slides were changed daily and the position of the truck readjusted. At the same time the upper surface of the stroma was dusted to remove any discharged spores which might have accumulated on it, and spores were wiped away from the lower surface of the transparent top of the casing around the stroma. Where an experiment was being conducted in the dark the necessary daily adjustments were made with the minimum of light from a weak electric hand-torch.

At the end of the day's run each slide was placed in a boiling-tube and the spores washed off with 10 ml. of hot water. Haemacytometer counts were made with this suspension. Each reading plotted in Figs. 2–5 represents the average of five counts. To convert this into the number of spores caught on each slide, the reading must be multiplied by 100,000.

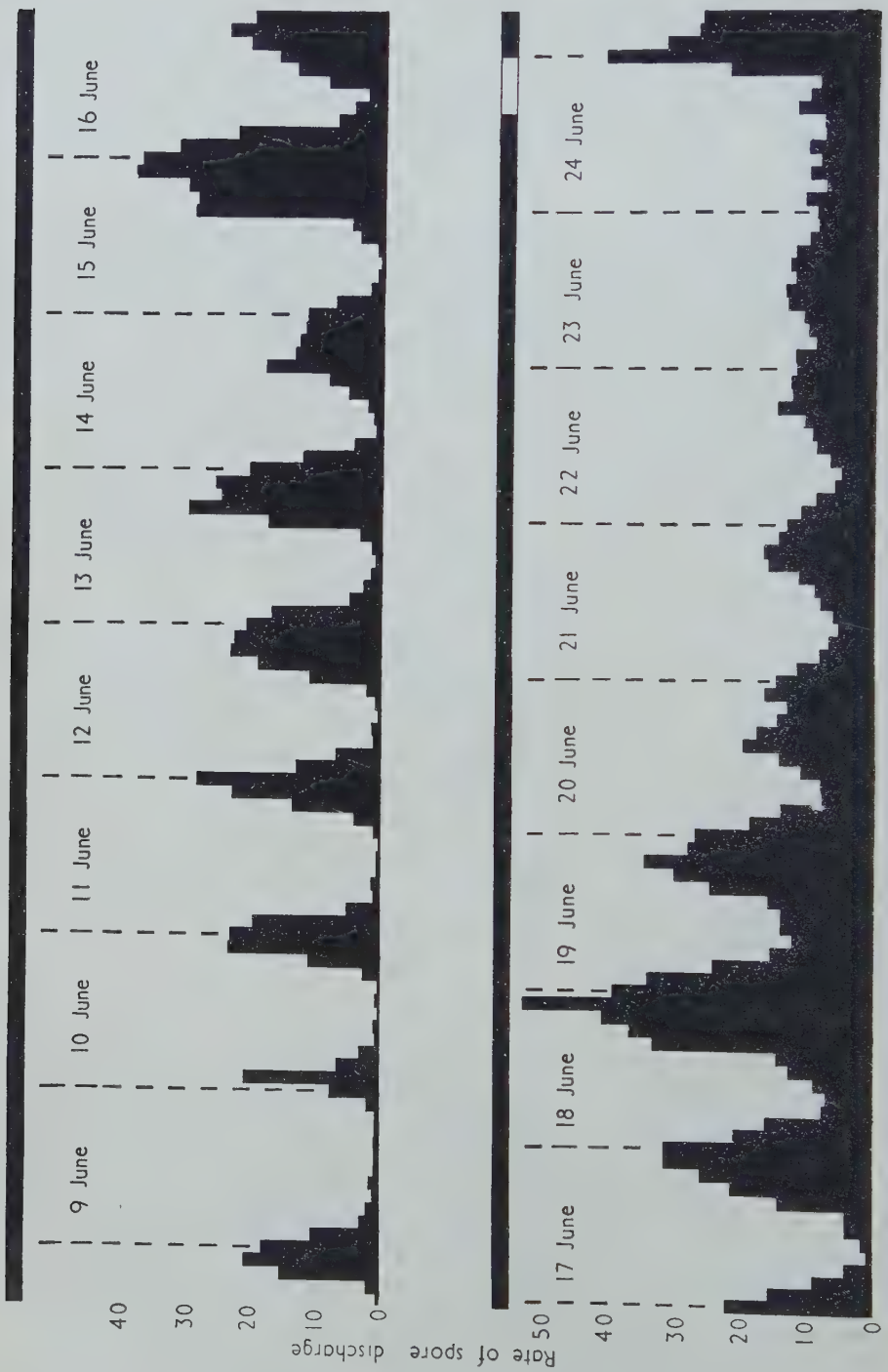
Most of the experiments were carried out in a specially constructed water-jacketed incubator which could be illuminated from above by four 40-Watt 'daylight' fluorescent tubes giving a light intensity of 100 foot-candles at the level of the fungus. Nevertheless, some experiments involving continuous darkness were performed in a dark-room with no temperature regulation. However, this room had an exceptionally uniform temperature during the period it was in use. In all experiments the temperature was $20{\cdot}5 \pm 1{\cdot}5°$ C., although the extremes of this range rarely occurred.

Since the stroma of *Daldinia* is black it might be expected that during experiments in the light absorption of radiant energy would lead to a significant rise of temperature. To test the magnitude of this effect a stroma was placed at the bottom of the light-incubator with the bulb of a thermometer embedded in its tissue. Near by was a wad of white cotton-wool of about the same size as the stroma and similarly provided with a thermometer. The light was switched on and observations of temperature were made at intervals, but after 4 hours and after 20 hours there was no significant difference between the readings of the two thermometers, which both corresponded with the general temperature of the incubator.

For each experiment a freshly collected stroma was used.

Results

In the first experiment the effect of continuous darkness on the daily march of spore liberation was studied. It will be seen (Fig. 2) that the rhythm of discharge continued with a maximum during the midnight hours and a minimum some 12 hours later. This pattern was maintained unmistakably for 12 days. However, during the last few days of this period (June 18–20), in spite of the striking contrast between spore output by day and by night, the actual rate of discharge during the minimum period was quite high, whereas during the earlier days in the dark (e.g. June 8–12) the daily minimum approached zero. After June 22 the periodicity appeared to have died out, and during June 23 and 24 the rate of spore liberation remained more or less steady. At 3 p.m. on June 25 the stroma was subjected to light which switched off at midnight,


9 June
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50
Rate of spore discharge

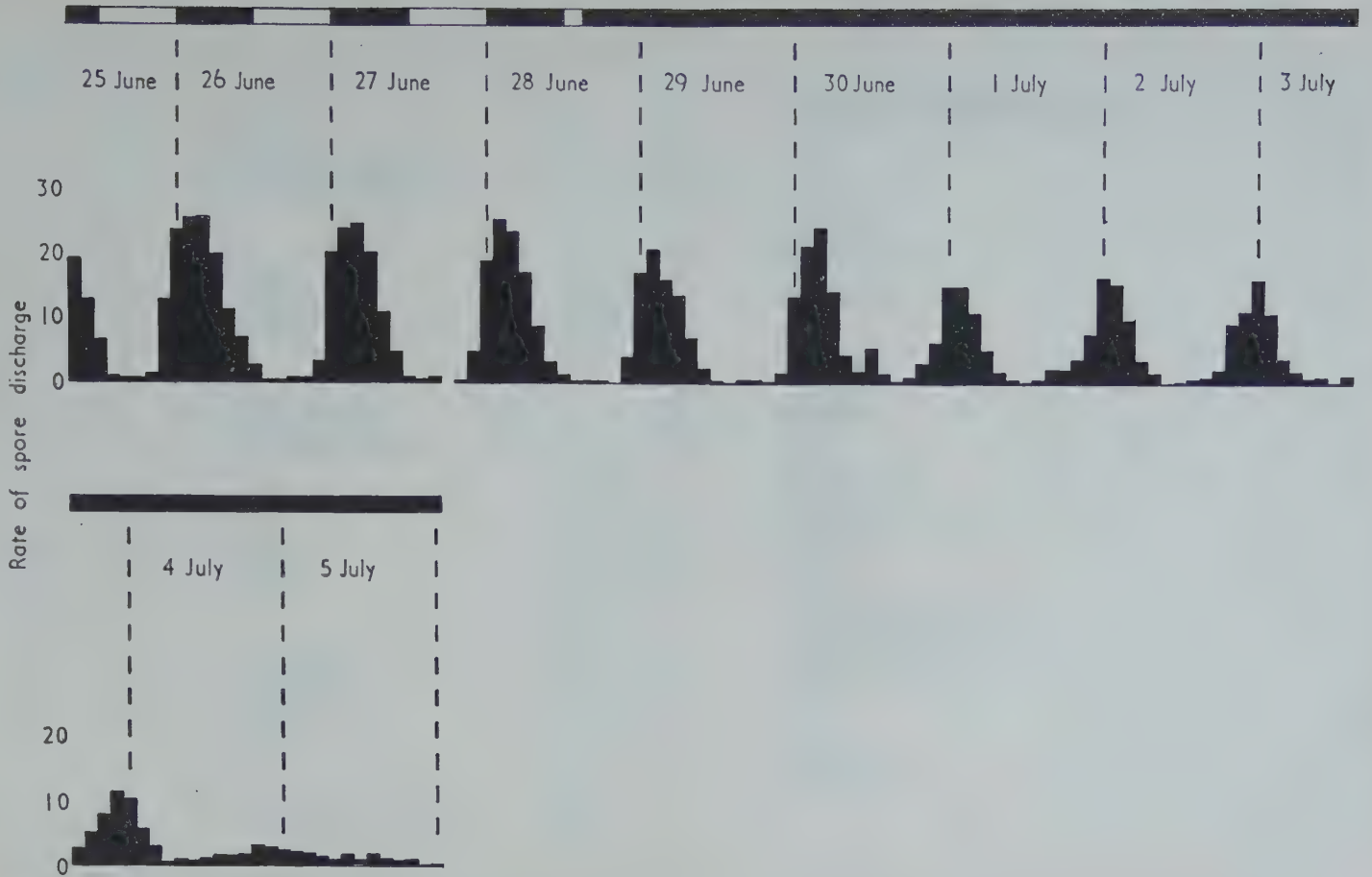

FIG. 2. Rate of spore discharge plotted against time. The horizontal strip above indicates conditions of illumination, black representing darkness and white representing illumination. Vertical interrupted lines give position of midnight.

and thereafter to alternating dark and light (midnight to noon, dark; noon to midnight, light). The introduction of this treatment had the immediate effect of re-establishing the periodicity of spore discharge. From the afternoon of June 28 the fungus was again subjected to continuous darkness. As before,

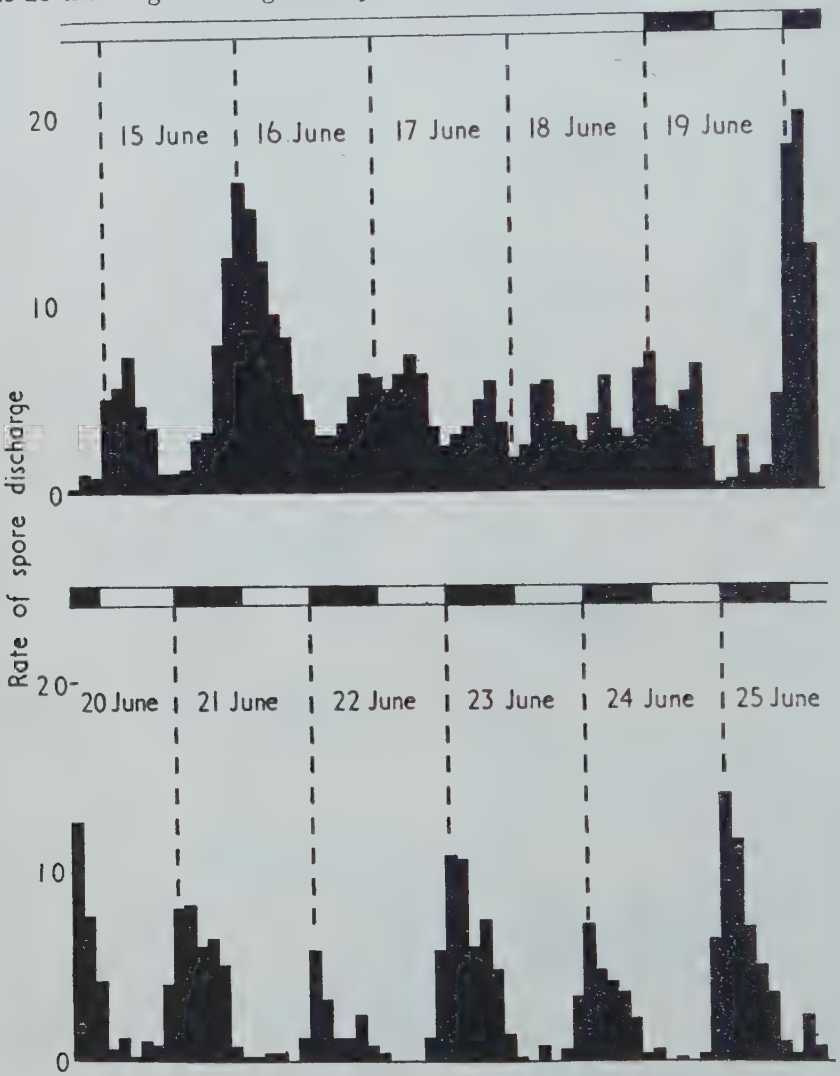

FIG. 3. Rate of spore discharge plotted against time. The horizontal strip above indicates conditions of illumination.

the nocturnal periodicity continued for the next seven days, but at the end of this time the rate of discharge was falling off and the experiment was discontinued.

In the next experiment (Fig. 3) the effect of continuous exposure to light was studied. For the first 2 days discharge was periodic with a nocturnal

maximum, but the peaks were not so pronounced as in the experiment in the dark (Fig. 2). During the third and fourth days the periodicity had disappeared and at 12.30 a.m. on June 19 the stroma was transferred to alternating conditions of darkness (12.30 a.m. to 12.30 p.m.) and light (12.30 p.m. to 12.30 a.m.). Soon after these conditions were established periodic spore discharge recommenced. It is to be noted, however, that, as in the previous experiment, discharge was not limited to the dark periods, but that the rate began to increase a few hours before the onset of each dark period. In this experiment the periodicity in continuous light disappeared much more rapidly than in the first experiment when continuous darkness obtained.

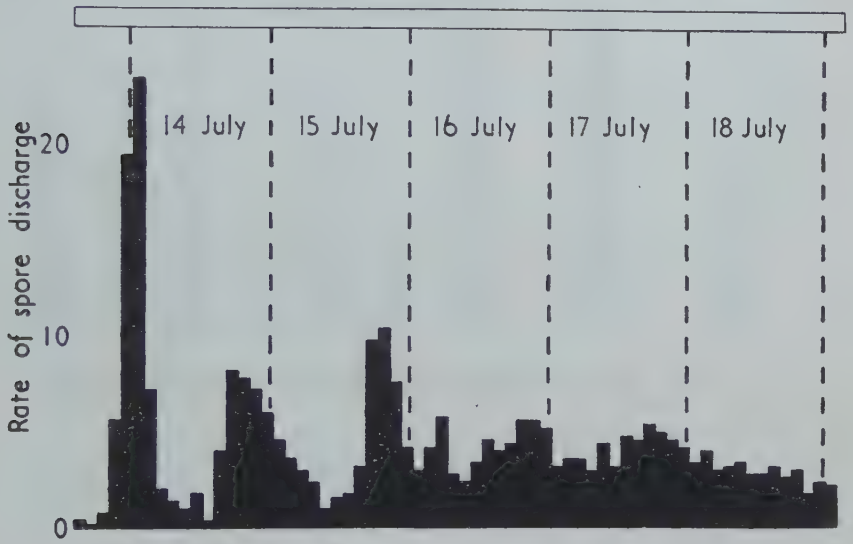

FIG. 4. Rate of spore discharge plotted against time. The horizontal strip above indicates conditions of illumination.

A further experiment (Fig. 4) gave the same result, periodic discharge lasting for only 2 or 3 days.

The final experiment reported here (Fig. 5) was concerned with an attempt to change the rhythm of *Daldinia* by subjecting a stroma to two separate light periods and two dark periods, all of the same duration, in the course of each day. The stroma was left for the first 2 days in a glass-fronted cupboard in a west-facing laboratory, and exhibited its natural periodicity. At 5 p.m. on July 7 it was transferred to the incubator and its first 6-hour dark period began. Thereafter the 6-hourly alternation of dark and light continued for the next 5 days. If for these days we consider the period from 5 p.m. to the following 5 p.m., it will be seen that instead of a single peak in the 24 hours, as in the first 2 days, there are now two, and these are associated with two dark periods. Further, this new distribution of spore-discharge rate occurred during the first day of treatment. The second peak induced in the 24-hour cycle was originally small relative to the first, but it gradually increased while

the first decreased until on the fourth and fifth days (5 p.m. July 10 to 5 p.m. July 11, and 5 p.m. July 11 to 5 p.m. July 12) they became almost exactly equal. After the 5-day period of this treatment the stroma was placed in continuous

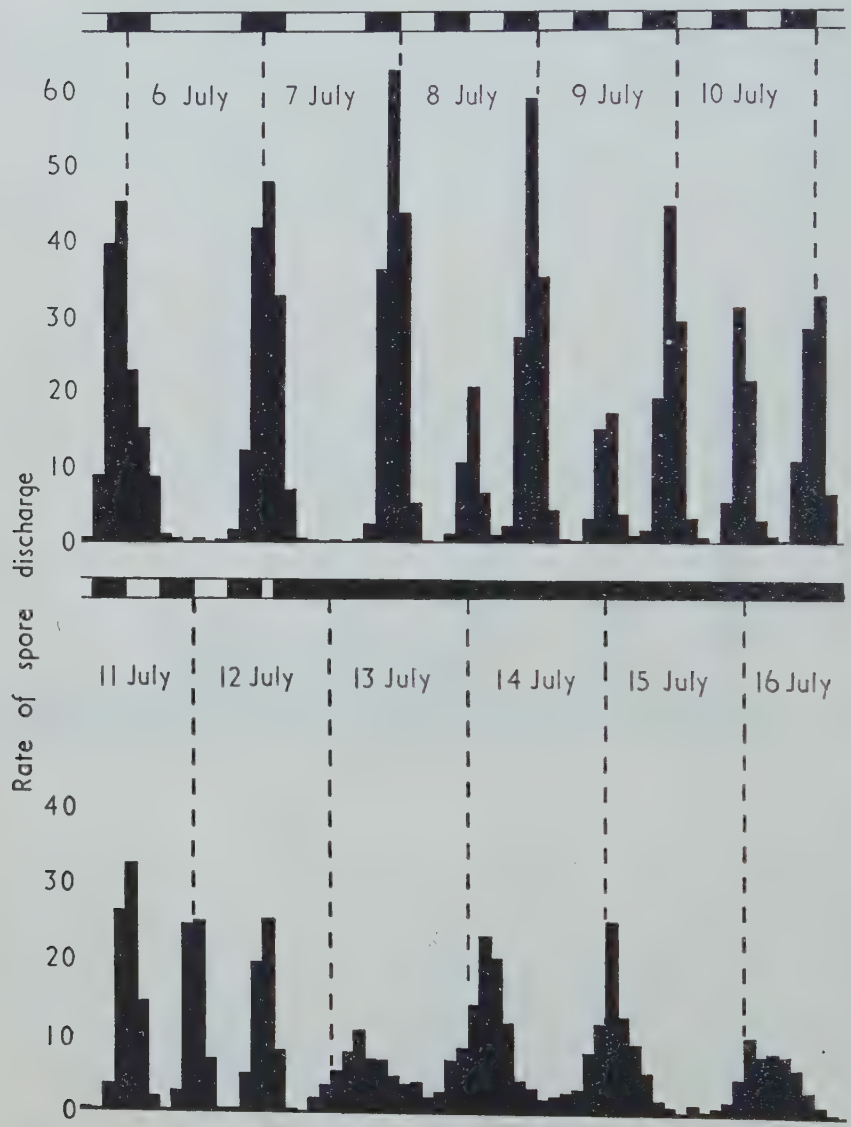

FIG. 5. Rate of spore discharge plotted against time. The horizontal strip above indicates conditions of illumination.

darkness. For the following 4 days discharge was periodic, but there was only one peak each day and this was associated with the midnight hours. It seemed that only the original rhythm was 'remembered'.

Discussion

The experiments reported above support the theory that alternation of light and darkness is concerned with the periodicity of spore discharge in *Daldinia*, since an alternation of 12-hour periods of light and darkness can re-establish the normal rhythm in a stroma in which it has died out either under the influence of continuous darkness or of continuous light, and also since it is possible to induce a new periodicity based on 6-hour instead of 12-hour periods of illumination and darkness.

It may well be, however, that other factors such as periodic changes in temperature might also induce a rhythm of discharge. This awaits investigation.

Again, the experiments reported here raise the question of what determines the continued periodic discharge of ascospores after the periodic conditions have ceased to operate.

It is clearly of interest to compare the behaviour of *Daldinia* not only with other fungi showing periodic discharge or ripening of spores, but also with the behaviour of other organisms which show daily rhythms of all kinds. However, discussion of this large problem is reserved until more is known about periodicity in *Daldinia* and other Ascomycetes.

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The Disappearance of Intermediates involved in Glucose Oxidation during the Starvation of the Fungus *Zygorhyncus moelleri*

BY

V. MOSES

(*Botany Department, University College, London*)

With three Figures in the Text

ABSTRACT

The respiration of carbohydrate-starved cells of *Zygorhyncus moelleri* showed a lag period of 2 to 3 hours when glucose was added before the rate of oxygen consumption became constant. Analysis of the rates of oxygen uptake from the time of addition of the sugar until they became constant showed that the lag period could not be ascribed entirely to a low concentration of free glucose in the cells in the period immediately following the addition of glucose to the medium. The analysis was, however, consistent with the supposition that the synthesis of an intermediary metabolite on the glucose oxidation pathway was necessary before the oxidation could proceed with a maximum speed. The length of the lag period could be reduced by adding extracts of cells together with glucose; extracts of unstarved cells were more effective than those of starved cells in shortening the lag period. Various known substances were also effective in this respect, acetate and ethanol being the most active. These results are discussed together with earlier work on the lag period.

IN an earlier paper (Moses, 1954) it was shown that when *Zygorhyncus moelleri* was grown on glucose and ammonium chloride, and then starved for 24 hours in a carbohydrate-free medium, the respiration rate increased slowly after the addition of glucose to the starved cells, and became constant only 2 or 3 hours after the sugar was added. Part of this lag period in the respiration could be ascribed to the stimulation of glucose oxidation by the presence of nitrogenous compounds remaining in the cells (Moses, 1954), and a small fraction to the time required for glucose to enter the cells (Moses, 1955). However, when nitrogen stimulation was prevented by the presence of a suitable concentration of azide or cyanide, or by starving the cells of nitrogen, most of the lag period when glucose was added still remained. It was shown (Moses, 1955) that the slow entry of glucose into the cells, resulting in a low internal glucose concentration, would not have limited the rate of respiration for more than about 11 per cent. of the total duration of the lag period.

Further investigation of this problem suggested that during starvation the concentration of one or more intermediates of glucose oxidation in the cells became the factor limiting the velocity of the whole reaction, and that the presence of the lag period could be associated largely with the accumulation of the metabolic intermediate(s) necessary before glucose could be oxidized at

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the maximum rate. The evidence for this proposition is presented in the present communication.

Materials and Methods

Growth and respiration measurements. The fungus was maintained on potato-dextrose agar slopes. An aqueous suspension of spores from agar slopes was inoculated into a glucose-ammonium chloride medium and the inoculated medium shaken in a water-bath at 25° C. for 18 hours. The cells were then centrifuged, washed, and suspended in phosphate buffer, pH 6·8. When starved cells were required these were shaken in phosphate buffer in the water-bath for 24 hours, and then centrifuged, washed, and suspended in fresh buffer.

An aliquot (2 ml.) of the cell suspension was pipetted into each of several Warburg micro-respirometer flasks. Carbon dioxide was absorbed by 0·2 ml. of 15 per cent. (w/v) potassium hydroxide in the centre wells, and the total volume of liquid in each Warburg flask (excluding the alkali) was always made up to 3·0 ml. with water.

Glucose was added to the cells as required from the side-arms to give a concentration in the medium of 0·4 per cent. (w/v) ($2{\cdot}22 \times 10^{-2}$ M.) unless otherwise stated. Organic acids, &c., when used together with glucose, had a concentration of $1{\cdot}11 \times 10^{-3}$ M. in the medium, and were adjusted to the appropriate pH before use. These techniques are described in detail by Moses (1954).

Preparation of cell-free extracts. Extracts of unstarved and starved cells were prepared by forcing the cell suspensions at very high pressures (20,000 lb. per square inch) through a small orifice to atmospheric pressure in the apparatus described by Milner, Lawrence, and French (1950) and French and Milner (1951). The whole assembly was cooled overnight in the refrigerator before use. The degree of disruption of the cells was complete; the extracts were not fractionated by centrifugation.

The cell-free extracts themselves had a negligible oxidative capacity with glucose as substrate.

Experiments

The synthesis of an intermediary metabolite. Starved cells were allowed to oxidize glucose for 3 hours, after which time they were centrifuged, washed, and suspended in buffer. This process greatly reduces the length of the lag period when a further quantity of glucose is subsequently added to the cells (Moses, 1955). Glucose was then added to the cells to give various initial concentrations in the medium, and the final constant respiration rate reached in each case was plotted against the initial concentration of glucose in the medium (Fig. 1). It will be seen from this curve that the enzyme system responsible for glucose oxidation appeared to be saturated when the external glucose concentration reached $1{\cdot}15 \times 10^{-2}$ M.

Glucose was next added to starved cells from the same growth batch not

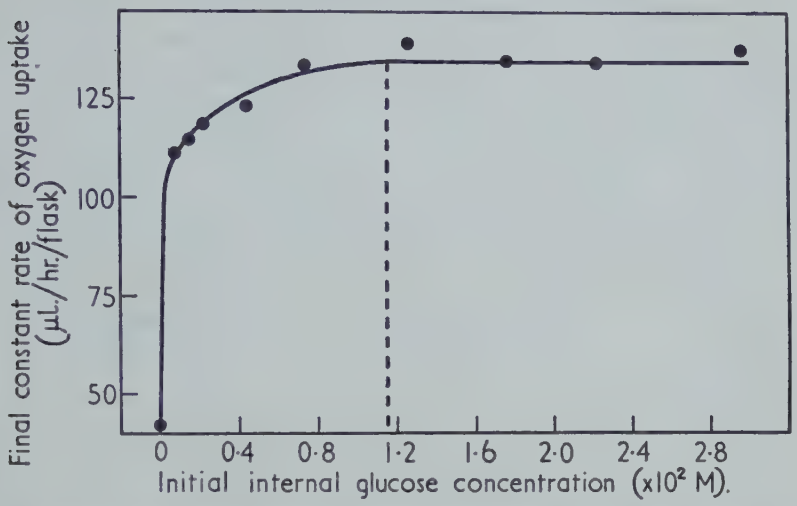

FIG. 1. Effect of the initial external glucose concentration on the final constant rates of oxygen uptake. The endogenous respiration has not been deducted: the rate of oxygen uptake in the absence of added substrate is therefore not zero. Each flask contained 2·8 mg. dry weight of cells suspended in 0·067 M. phosphate buffer, pH 6·8.

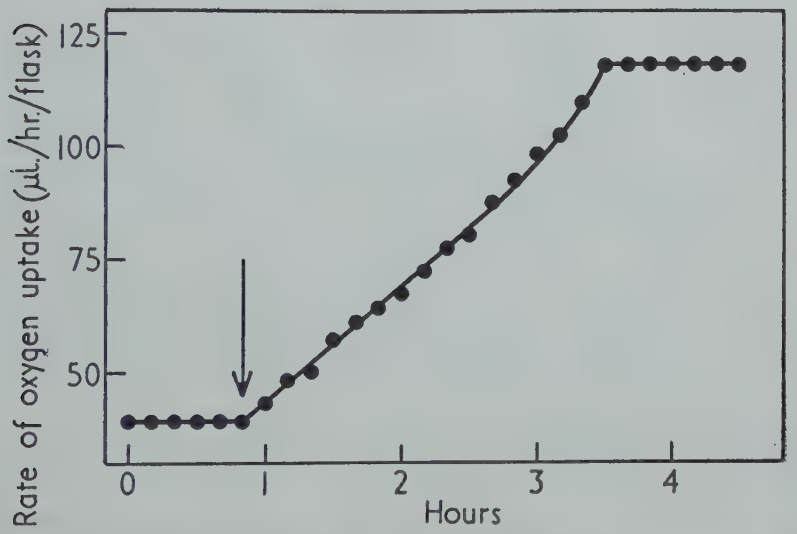

FIG. 2. Rates of oxygen uptake following the addition of glucose to starved cells. Initial glucose concentration: $2{\cdot}22 \times 10^{-3}$ M. (i.e. 6·67 μ moles of glucose added). Glucose added as indicated by the arrow. The flasks contained 2·8 mg. dry weight of cells in 0·067 M. phosphate buffer, pH 6·8.

pretreated with glucose to give an initial concentration in the medium of $2{\cdot}22 \times 10^{-3}$ M. (which was below the saturation level), and the rate of oxygen uptake was plotted against time until it became constant (Fig. 2).

It was assumed that the effective concentration of glucose inside the cells

controlled the degree of enzyme saturation and was the limiting factor for the rate of oxygen uptake. It was thus possible to draw a hypothetical curve for the apparent effective intracellular glucose concentration after glucose had been added to the medium by interpreting the rates of oxygen uptake at intervals as being the direct results of certain internal glucose concentrations. It has already been shown that when the rate of oxygen uptake becomes constant the intra- and extra-cellular glucose concentrations are approximately equal, and that the former never exceeds the latter (Moses, 1955).

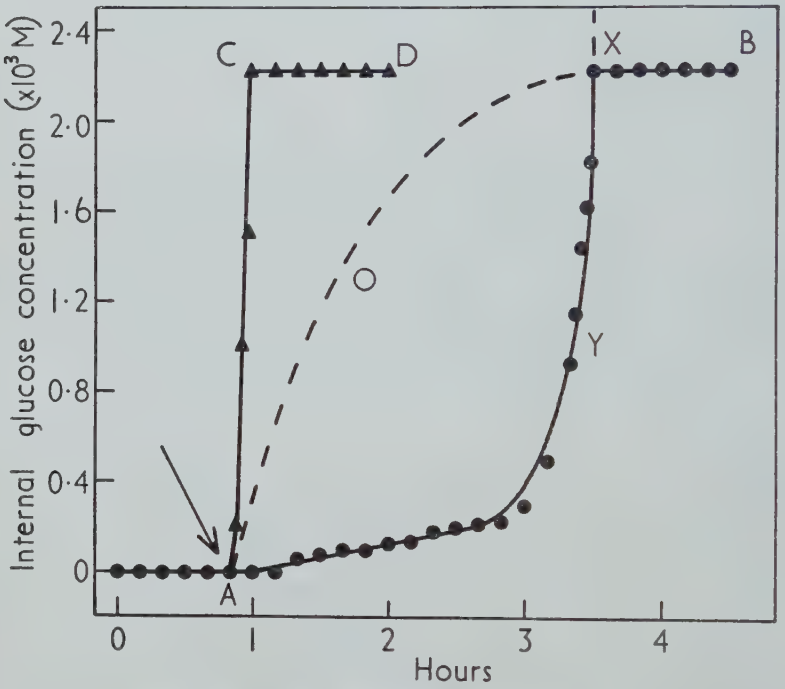

FIG. 3. Apparent intracellular glucose concentration calculated from the data of Figs. 1 and 2. *AYXB*: *Z. moelleri*; *ACD*: baker's yeast. Glucose added as shown by the arrow. For further details see text.

Fig. 3 shows the calculated curve for the development of the intracellular glucose concentration in *Z. moelleri*, and for comparison a curve derived in a similar manner for baker's yeast is also shown. With the latter organism the addition of glucose to starved cells resulted in a constant rate of respiration being attained within 10 to 15 minutes, and little or no lag period was manifest.

From the curve *AYXB* for *Z. moelleri* (Fig. 3) it can be seen that the intracellular glucose concentration was apparently very low for a long period as compared with yeast, and suddenly rose rapidly to a constant maximum level. The shape of this curve made it appear most unlikely that this represented the true development of the intracellular glucose concentration, particularly as earlier work on the entry of glucose into the cell had suggested that the development of the intracellular concentration followed a curve similar in form

to that of the line *AOXB* in Fig. 3 (Moses, 1955). The discontinuity of the curve *AYXB* at the point *X* rather suggests that the curve is really the resultant of two curves, one *AYX* and the other *XB*, representing the limiting of the rate of respiration by different factors at different periods.

The line *XB* represents the level of glucose added to the medium, and from previous evidence it is known that when the rate of oxygen consumption becomes constant the internal and external glucose concentrations are approximately equal. Over the range *XB* the glucose concentration in the medium limited that in the cells (and hence the rate of respiration), and the development of the glucose concentration within the cells might be represented roughly by the line *AOXB*. It seemed possible that over the range *AYX* the factor limiting activity would not be the glucose concentration but perhaps the concentration of some intermediary metabolite which disappeared from the cells during starvation and was resynthesized during the lag period, the extent of the synthesis being represented by the line *AYX*.

Using this as working hypothesis, investigations were undertaken to examine further the synthesis of a metabolite during the lag period.

The effect on the lag period of pretreatment with varying quantities of glucose. When a small quantity of glucose was added to starved cells the rate of oxygen uptake rose slowly to a constant rate and after some time returned to the endogenous value, due to exhaustion of the glucose in the medium (Moses, 1954). If a second similar amount of glucose was then added to the cells the rate of oxygen uptake rose to the same constant rate as before, but the lag period before the constant rate was reached was very much shorter (Moses, 1955).

In order to find how much glucose had to be added at the first addition in order to shorten the subsequent lag period, the amount of the first quantity of glucose added was varied (0·44 to 6·67 μ moles), whilst the second quantity remained always the same (66·7 μ moles). As very small quantities of glucose were used at the first addition the lag period was not complete by the time the glucose was exhausted. It was found that the larger the first quantity of glucose, the longer was the first lag period but the shorter the second lag period; the sum of the two lag periods was always approximately the same (Table I), and was equal to that observed when one large quantity (66·7 μ moles) was added without pretreatment with a smaller one.

This showed that appreciable amounts of glucose had to be present for a definite and considerable period before the rate of oxygen uptake became constant at its maximum value. Presumably, therefore, glucose was stimulating a reaction that depended on its continued presence, as distinct from a 'trigger' reaction. Such a reaction is consistent with the suggestion that in the continued presence of the substrate an intermediate on the metabolic pathway is synthesized, thus completing the reaction chain for the metabolism of the substrate.

Diffusion of a factor out of the cells during starvation. Starved cells were incubated with several substances prior to the addition of glucose. These substances included adenosine triphosphate, magnesium ions, riboflavin, and a

concentrate of the medium in which the cells had been starved (which was prepared by evaporation at room temperature *in vacuo*); no reduction of the length of the lag period was obtained with any of these substances. The absence of any effect with the concentrated starvation medium precludes the possibility that the disappearance of an intermediary metabolite involved diffusion out of the cells during starvation, but offers no evidence on the question of the breakdown of the metabolite within the cells. In the latter instance it is quite possible that such a metabolite would not diffuse into the cells in any case, so that supplying it to the medium would not necessarily make it available to the relevant sites inside the cells, and thus would not shorten the lag period.

TABLE I

The Duration of the First and Second Lag Periods when Glucose was added to Starved Cells in Two Successive Quantities

Glucose was added to the cells in two quantities, the second not being added until the first had been completely metabolized. The first quantity was varied in size; the second quantity was always the same. Each flask contained 3·7 mg. dry weight of cells in 0·067 M. phosphate buffer, pH 6·8.

1st glucose addition (μ moles).	1st lag period (min.).	2nd glucose addition (μ moles).	2nd lag period (min.).	Sum of the 2 lag periods (min.).
0·44	15	66·7	92	107
1·11	26	66·7	83	109
1·66	26	66·7	80	106
2·22	40	66·7	72	112
6·67	64	66·7	45	109
66·70	114	—	—	114

Addition of extracts of cells in different states of starvation. Cell-free extracts of starved and unstarved cells were prepared by disintegration by the high-pressure method mentioned earlier. The extracts were added to whole starved cells simultaneously with glucose, and the length of the lag periods compared with the lag period when glucose was added alone. As the extracts contained nitrogenous matter a comparison was also made with the lag period when glucose was added together with ammonia. The results are thus complicated by the stimulation of glucose oxidation by nitrogenous substances (which lengthened the normal lag period as well as increased the final rate of oxygen uptake, Moses, 1954), and by the extracts themselves containing oxidizable material. In order to evaluate the effect of these cell-free extracts on the lag period shown by starved cells, the criterion used was the ratio, final rate of oxygen uptake in μl./hr. (I): total length of the lag period in minutes (II).

Experiment A (Table II) showed that unstarved cells were more effective than starved cells in stimulating a relative increase in respiratory activity. This suggested the presence of a factor in unstarved cells which was present to a lesser extent in starved cells. Further, whereas the starved-cell extract reduced the lag period for glucose oxidation from 175 minutes (when glucose was

added alone) to 120 minutes, and resulted in a final rate of oxygen uptake of 312·5 μl. per hour, the unstarved cell extract was able to stimulate the rate of oxygen uptake to this value in 100 minutes. There was therefore a quantitative difference between starved and unstarved cells which could be correlated with the disappearance of an intermediate during starvation.

The relatively small difference between the effects produced by the two extracts was probably associated with the short starvation period used (3 hours)

TABLE II

Effect on the Length of the Lag Period of adding Cell-free Extracts of Starved and Unstarved Cells to Starved Cells together with Glucose

About 4 mg. dry weight of starved whole cells were used per flask and an equivalent quantity of extract added as indicated in the table to each flask. The medium was 0·067 M. phosphate buffer, pH 6·8.

Substances added to starved cells.	I. Rate of oxygen uptake (% of control).	II. Lag period (% of control).	I/II.
Experiment A			
Glucose	55·5	86·2	0·64
Glucose+ammonium chloride	100·0	100·0	1·00
Glucose+disrupted unstarved cells	164·7	73·4	2·24
Glucose+disrupted starved cells	119·3	59·1	2·02
Experiment B			
Glucose	57·9	51·5	1·12
Glucose+ammonium chloride	100·0	100·0	1·00
Glucose+supernatant from whole unstarved cells boiled for 7 minutes	67·5	59·3	1·14
Glucose+disrupted unstarved cells	82·7	37·1	2·23
Glucose+disrupted unstarved cells boiled for 7 minutes	81·1	99·4	0·82
Glucose+disrupted unstarved cells boiled 7 minutes with N-HCl and neutralized	84·5	57·5	1·47

for the starved cell extract. This short period was necessary in order to be able to use extracts from cells of the same growth batch in one experiment, and so to be able to use identical quantities of each.

Experiment B (Table II) showed that the factor present in unstarved cells could not be extracted efficiently from whole cells by boiling alone, and that even with disrupted cells the extract was less effective after boiling. Acid hydrolysis of the extract (with subsequent neutralization) did not greatly reduce the efficacy of the factor, and it seemed that this was thermostable but may have been adsorbed on the precipitated protein after boiling.

In expt. B there was a lag period of 86 minutes when glucose was added alone to the starved cells, and the final rate of oxygen uptake was 187 μl. per hour. When 0·5 mg. of ammonium chloride was added together with the glucose the same rate of oxygen uptake was reached in 50 minutes, and with glucose and an extract of unstarved cells in 12 minutes.

Effect of pre-incubation with other substrates on the glucose oxidation lag period. Starved cells were incubated with various sugars, glycerol, glycine, citrate, and acetate before glucose was added. Some of these substrates were oxidized by the cells (Table III), and in such cases also showed lag periods. Other substances (xylose, glycerol, glycine, and citrate) were oxidized very slowly or

TABLE III

Addition of Various Substances to Cells followed by a Subsequent addition of Glucose

Glucose concentration: $2{\cdot}22 \times 10^{-3}$ M. The concentration of the other substances was such as to give a concentration of carbon equivalent to this concentration of glucose. Each flask contained 3·9 mg. dry weight of cells in expt. A and 2·0 mg. in expt. B. The medium was 0·067 M. phosphate buffer, pH 6·8.

Substance added before glucose.	Lag period (min.).	Final rate of oxygen uptake (μl./hr./flask).	Subsequent glucose addition: Lag period (min.).	Subsequent glucose addition: Final rate of oxygen uptake (μl./hr./flask).
Experiment A				
None (endogenous respiration)	—	25	—	—
Acetate	70	232	30	138
Galactose	80	46	30	125
Glucose	70	168	22	162
Sucrose	60	87	28	146
Experiment B				
None (endogenous respiration)	—	12	—	—
Citrate	—	12*	60	46
Glucose	123	77	17	78
Glycerol	—	12*	62	35
Glycine	15	19*	70	35
Xylose	114	22	37	57

* i.e. little or no oxidation.

not at all under the experimental conditions used. Nevertheless, the addition of glucose subsequent to the incubation of the cells with all these substrates resulted in a reduction of the lag period compared with that when glucose was added alone. The glucose concentration was $2{\cdot}22 \times 10^{-3}$ M. and that of the other substrates such as to give a concentration of carbon in the medium equivalent to that present with the above concentration of glucose.

Pre-incubation with glucose, sucrose, or acetate was the most effective means of reducing the lag period when glucose was later added.

Addition of various substances together with glucose. Various organic substances were added to starved cells together with glucose, and the final oxidation rates and the lag periods were examined. The glucose concentrations were $2{\cdot}22 \times 10^{-2}$ M. and that of the other substances $1{\cdot}11 \times 10^{-3}$ M. The substances were all adjusted to a definite pH before being added to the cells.

The effect of these substances in shortening the lag period (Table IV) divides them into three groups:

(i) ineffective: citrate, oxalacetate, DL-lactate, DL-malate, and (probably) fumarate and pyruvate;

(ii) slightly effective: fructose 1,6-diphosphate, and succinate;

(iii) very effective: acetate and ethanol.

TABLE IV

Effect on Lag Period of the Addition of Various Substances together with Glucose at various pH Values

Glucose concentration: $2{\cdot}22 \times 10^{-2}$ M. Concentration of other substances: $1{\cdot}11 \times 10^{-3}$ M. Composite results from several experiments. Medium contained 0·067 M phosphate buffer of the pH indicated.

Substance added together with glucose.	pH.	Rate of oxygen uptake (as % of control with glucose alone).	Lag period (as % of control with glucose alone).	Whether substance is oxidized as sole substrate.
None	4·3–6·8	100·0	100·0	—
Acetate	4·3	107·3	39·5	Yes, in very small quantities*
„	5·0	99·5	29·4	
„	6·8	100·5	50·2	Yes
„	6·8	98·4	55·7	
Citrate	5·0	99·5	108·0	No
Ethanol	6·8	94·6	39·6†	Yes
Fructose 1,6-diphosphate	5·0	107·1	83·7	No
Fumarate	5·0	105·7	96·0	Slightly
DL-lactate	5·0	102·4	105·6	Slightly
DL-malate	5·0	100·8	102·4	Slightly
Oxalacetate	5·0	100·6	99·3	Very slightly
Pyruvate	4·3	96·1	97·8	No
Succinate	4·3	106·1	89·0	Slightly
„	5·0	104·1	88·8	

* Inhibitory in higher concentrations: see text.

† Ethanol, being volatile, diffused over to some extent from the side-arm of the Warburg flask into the main compartment before the flask was tipped. A high 'endogenous' rate of respiration was observed. Accordingly the value of 39·6 per cent. in the presence of ethanol must be taken with caution.

When used singly as substrates (i.e. without glucose), citrate, pyruvate, and fructose 1,6-diphosphate were not oxidized at all; fumarate, succinate, DL-lactate, and DL-malate were oxidized very slowly, and oxalacetate still more slowly. The low degree of oxidation of these acids may have been due to a low ability to penetrate into the cells except at low pH. Barron, Ardao, and Hearon (1950*a*), working with baker's yeast, found that citrate and succinate were oxidized in acid solutions up to pH 4 only, and malate and α-ketoglutarate were not oxidized, undoubtedly because of lack of penetration. Beevers,

Goldschmidt, and Koffler (1952) also mention the difficulty of penetration of many important substrates which are weak acids.

Further work at pH 3·5 showed that at this lower pH pyruvate, succinate, and α-ketoglutarate, though not citrate, would penetrate the cell membrane and become oxidized. Their effect on reducing the lag period when added simultaneously with glucose was correspondingly enhanced (Table V).

Acetate and ethanol, however, were oxidized very rapidly by *Z. moelleri*. Acetate was very effective also, when used in low concentrations, for shortening the lag period for glucose oxidation. When used as the sole substrate at higher concentrations ($6{\cdot}67 \times 10^{-2}$ M.) it was oxidized rapidly at pH 6·8, but was not oxidized below pH 6·3. At pH 4·3 it inhibited the endogenous respiration.

TABLE V

Effect on Lag Period of the Addition of Acetate, Pyruvate, Succinate, Citrate, and α-Ketoglutarate together with Glucose at low pH

Glucose concentration: $2{\cdot}22 \times 10^{-2}$ M. Concentration of other substances: $1{\cdot}11 \times 10^{-3}$ M. Each flask contained 5·51 mg. dry weight of cells. The medium contained 0·067 M. phosphate buffer, pH 3·5.

Substances added.	Rate of oxygen uptake (μl./hr./flask).	Lag period (min.).	Lag period (as % of control with glucose alone).
None (endogenous respiration)	30	—	—
Glucose	179	111	100·0
Glucose+acetate	181	57	51·4
Acetate	145	—	—
Glucose+pyruvate	160	67	60·4
Pyruvate	36	—	—
Glucose+succinate	167	80	72·1
Succinate	48	—	—
Glucose+citrate	181	120	108·1
Citrate	28	—	—
Glucose+α-ketoglutarate	188	105	94·6
α-Ketoglutarate	50	—	—

Ajl (1952) and Barron *et al.* (1950*b*) have found with *Escherichia coli* and *Corynebacterium creatinovorans* respectively that the optimum pH for acetate oxidation was 6·5 to 7·5 and that no oxidation took place below about pH 5·0. Mickelson and Schuler (1953) found no oxidation of acetate by *Ashbya gossypii* at pH 2 to 3; the optimum for this oxidation was pH 5.

Tang (1937), using *Chlorella pyrenoidosa*, found a similar inhibition at low pH by lactic acid. *C. pyrenoidosa* oxidized lactic acid at high pH, but at a similar concentration the acid was inhibitory to respiration, both in the presence and absence of glucose, at pH 5·4. The author ascribed the effect of lactic acid to an increase in internal hydrogen-ion concentration, and a similar explanation may be valid for the effect of acetic acid on *Z. moelleri* at low pH.

The large reduction in the glucose oxidation lag period produced by acetate

and ethanol suggests that these substances can enter the cells rapidly and either complete the missing links in the glucose oxidation pathway themselves or be rapidly converted into substances which can do so. Conway and Downey (1949) have suggested that acetate is one of the substances which can penetrate rapidly into the central regions of baker's yeast cells, and this agrees with its rapid effect on *Z. moelleri*.

Discussion

Lynen (1942) investigated fully a lag period of several hours in the rate of oxygen uptake when acetate was added to starved baker's yeast. The lag period could be reduced in appropriate circumstances by glucose, ethanol, propanol, butanol, pyruvate, succinate, and most effectively by succinic acid semialdehyde (β-aldehydopropionic acid). Lynen suggested that acetate was oxidized via the Krebs tricarboxylic acid cycle in which the first reaction was the condensation of acetate with oxalacetate to form citrate, and that this reaction was coupled with the oxidation of an aldehyde to an acid. Glucose, ethanol, propanol, and butanol could all give rise to aldehydes and reduce the lag period. Succinate and pyruvate reduced the lag period by supplying oxalacetate, and succinic acid semialdehyde did so by a combination of both these processes. Lynen concluded that 'the lag period in the breakdown of acetic acid in starved yeast is associated with the low metabolic turnover in such a yeast, so that some time is required for the production of sufficient oxalacetic acid and aldehyde from reserve material'.

There is not as yet sufficient evidence to come to such a definite conclusion with regard to the lag period for glucose oxidation shown by starved *Z. moelleri*. Several substances, particularly acetate, were able to reduce the lag period for glucose oxidation; these substances are associated with the Krebs cycle mechanism. The comparatively large effect of acetate was probably due to its great ability to penetrate rapidly into the cells, compared with a much lower ability for penetration of the other organic acids tested. Thus, although it appears that the lag period can be ascribed mainly to the building up of a supply of an intermediate metabolite, the localization of this substance is not so clear as in acetate oxidation by baker's yeast. The nature of the substances which were able to reduce the lag period suggests that the deficiency might have occurred in an organic acid cycle of the di- or tri-carboxylic acid type.

Another possible interpretation of the lag period is that it might be due to a carbohydrate-sparing effect. Carbohydrate oxidation might only proceed at a maximum rate when the concentration of some cellular component, such as lipid, is at an optimum level. Were this level to fall during starvation, a fresh supply of sugar would be used first to restore this level before rapid oxidation commenced. Certain observations indicate that this is not the case. In the first place, lag periods of up to several hours' duration have been observed with *all* substrates tested which are oxidized by this organism after starvation; these included several sugars and organic acids. The sparing effect would therefore have to apply to numerous substances. Secondly, if the

concentration of a depleted cell constituent had to be replaced from its starvation level to a higher level before oxidation occurred at a maximal speed, a constant amount of external substrate would be required to do this, so that the percentage of external substrate which was completely oxidized would not be constant, but would become greater as more substrate was supplied. It has been shown, however, that assuming, as seems probable, that the endogenous respiration continues during the metabolism of an external substrate, the percentage of added glucose completely oxidized was very constant, and exhibited no increase as the amount of glucose supplied was increased: when 0·44, 1·11, 1·67, 2·22, and 6·67 μ moles of glucose were supplied, the percentage completely oxidized was 16·7, 18·1, 17·4, 18·5, and 16·7 respectively (Moses, 1955).

Furthermore, were a sparing effect responsible for the lag period, in the time immediately following the addition of the sugar to the cells the fraction of the sugar 'assimilated' (i.e. neither found in the cells nor the medium as free glucose, nor accounted for by oxidation) compared with the fraction oxidized would be greater than after the termination of the lag period, in order to restore the level of the unknown component necessary for maximal oxidation. Previous work (Moses, 1955) has shown, however, that the ratio of glucose assimilated to glucose oxidized *rises* during the lag period to a maximum and constant value at the end of the lag period; the utilization of glucose (as measured by the disappearance of free glucose from the complete system, including the cells) shows a lag period similar in duration to that exhibited by the rates of oxygen consumption and carbon dioxide evolution. Owing to the catalytic amounts involved, these arguments would apply much less readily to the synthesis of metabolic intermediates during the lag period.

The interpretation of the appearance of the lag period as largely due to the synthesis of an intermediary metabolite throws light on the phenomena observed in connexion with cell permeability in this organism (Moses, 1955). It was shown that when two successive small quantities of glucose were added to cells, the second quantity not being added until the first had all been metabolized, the second lag period was much shorter than the first. In the light of the present work it becomes possible to interpret this fact by supposing that after the oxidation of the first quantity of glucose the cells had sufficient supplies of the intermediate to allow the second quantity to be oxidized rapidly with very little delay. It was also found that the longer the period elapsing between the complete utilization of the first quantity of glucose and the addition of the second quantity, the longer was the second lag period. This can also now be explained as the increasing depletion of the metabolite supplies as starvation proceeded.

Together with the earlier results reported (Moses, 1954, 1955) it is now possible to ascribe the appearance of the lag period for glucose oxidation in starved *Z. moelleri* to three main factors. In order of significance these are: resynthesis of an intermediary metabolite; stimulation of respiration by a source of nitrogen within the cells; and the entry of glucose into the cells.

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Heterophylly in some Species of *Callitriche*, with especial reference to *Callitriche intermedia*

BY

HERBERT JONES

(*Department of Botany, University College of Wales, Aberystwyth*)

With Plate II and four Figures in the Text

Abstract

Callitriche stagnalis, *C. obtusangula*, and *C. intermedia*, in that order, were found to show a progressively greater variation in leaf form. Axes of *C. intermedia* bearing crowns of ovate leaves were submerged under various light and temperature conditions and the growth rates studied in relation to the form of the leaves produced. Leaf movements were also studied in connexion with the growth of such submerged shoots and with the general question of heterophylly.

Introduction

A LEAF, from the time it appears as a protuberance below the shoot apex until it attains maturity, shows a gradual change in form. This leaf, once its final form is attained, may differ from other mature leaves on the same plant. The differences in form of mature leaves fall into two main categories. In what is probably the larger group there is a change in leaf form during the development of the plant to maturity. *Callitriche stagnalis* is an example in which the juvenile leaves are linear, the adult leaves being ovate. In the second group the leaves of the mature plant show heterophylly, as in

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the case of *C. intermedia*. Here linear or ovate leaves may be found, according to certain conditions of the environment.

In a study of heterophylly the genus *Callitriche* is of special interest because the plasticity of leaf form varies considerably in different species. This is of considerable ecological significance since the wider range of leaf forms of *C. intermedia*, for instance, enables it to live in a wider range of aquatic habitats than *C. stagnalis*.

In a review of the literature of heterophylly, Ashby (1948) comments upon the change in emphasis in the treatment of the subject. The classical view stressed the study of developmental morphology in relation to problems of phylogeny, whereas the modern approach has become concerned chiefly with causal morphology. In the latter field Goebel (1900) showed that the juvenile form of leaf in some plants was often associated with unfavourable conditions. Under conditions of low light intensity, for instance, persistence in or reversion to the juvenile form of leaf could be effected. This important concept has had considerable influence on theories of heterophylly ever since (Arber, 1920).

The mechanisms involved in changes of leaf form have attracted much attention. A great deal of the work has been concerned with aquatic plants because of the wide variety of leaf form which is characteristically displayed in the aquatic environment and also because the problems of study are simpler in the comparatively uniform water medium. Many aquatics display a different (often juvenile) form of leaf when submerged from that formed on the surface of the water. Åberg (1943) has shown that in *Lobelia dortmanna* the longer leaves formed at greater depths of submergence are correlated with the lower light intensities. McCallum (1902), however, has related the ovate aerial leaf in *Proserpinaca palustris* to withdrawal of water from the plant tissues through exposure to the air. Burns (1904) queried McCallum's results, claiming that light was the factor responsible for the leaf change. McCallum's methods have been applied by the present writer in the case of *Callitriche intermedia* (Jones, 1952).

The variation in the leaf form of aquatics is by no means always related to submergence or emergence. In *Potamogeton perfoliatus* Pearsall and Hanby (1925) showed that the leaf form was dependent on the calcium/potassium balance in the nutrient solution. In *Sesamum orientale* a 14-hour or longer photoperiod produces an ovate, serrate leaf while linear-lanceolate leaves are formed under short-day conditions (Sen Gupta and Payne, 1947). Allsopp (1952, 1953) found that under conditions of inadequate nutrition *Marsilea* sp., grown in aseptic culture, produced leaves much simpler in form than those normally found and that the reversion to the juvenile leaf form could be effected by inadequate nutrition.

In the present work the question of heterophylly in *Callitriche intermedia* is considered in relation to the growth of the submerged shoot. In particular, this growth is studied in relation to the leaf form under different conditions of light and temperature. In this connexion the work of Juhren and Went

(1949) is of special interest. These workers found that the lack of development of *Squash* leaves in darkness was not associated with lack of light in the nutritional sense. By injecting the petioles with sucrose solution (with sulphanilamide to prevent growth of micro-organisms) the plants were kept alive for periods up to 30 days. Stem elongation was marked during this period. Leaf primordia were initiated at about the same rate as for plants in light, but the leaf blade ceased development at an early stage. Nightingale (1933) studied the growth of *tomato* plants under different conditions of temperature but at a constant light intensity. At 35° C. several abnormal features became evident, the plants being quite normal at lower temperatures. While Nightingale made no comment on this, several of the characters shown by the plants grown at 35° C. resemble those of etiolated plants.

In general, it appears that stem growth is less during the day than at night (Burkholder, 1936). While in many cases this may be due to some external factor, in the present investigation it is shown that diurnal fluctuation in the growth rates of submerged *Callitriche intermedia* shoots is independent of both light and temperature. In this particular case the facts point to the existence of an endogenous diurnal periodicity in the growth rate. One of the more conspicuous examples of diurnal periodicity is the leaf movements of some plants, although, owing to the coincidence of the movements with diurnal fluctuations in light and temperature, the nature of the phenomenon has tended to be overlooked. Bünning (1948) showed that two well-marked groups of plants may be separated, those in which the leaves rise at night and those whose leaves rise towards midday. Bünning showed that the former category were long-day plants and the latter were short-day plants. Since the leaves of a *Callitriche intermedia* shoot (submerged from the floating state) show leaf movements, and since these movements may be correlated with the daily rhythm of growth, the actual mechanical basis of the leaf movement is of considerable interest. Little appears to be known of this apart from the work of Yin (1941). In *Carica papaya* the leaf movement was associated with differential distribution of auxin in the adaxial and abaxial surfaces of the petiole at different times of the day.

The first part of the present work describes an investigation into the degree of heterophylly shown by *Callitriche intermedia*, *C. stagnalis*, and *C. obtusangula*, the latter species occupying an intermediate position as regards variation in form of the mature leaf. The second part of the investigation concerns two features of *C. intermedia* which are characteristic of the submergence of an axis which has previously borne a crown floating at the surface. The first feature is the high rate of growth of the axis on submergence, the second the movements of the leaves which occur following submergence.

Terminology

In the following description a crown of linear leaves on the shoot is referred to as a linear crown and a crown of ovate leaves as an ovate crown.

Transplant Experiments with, and Description of Seedlings of, *Callitriche intermedia*, *C. obtusangula*, and *C. stagnalis*

(*a*) *Transplant experiments*

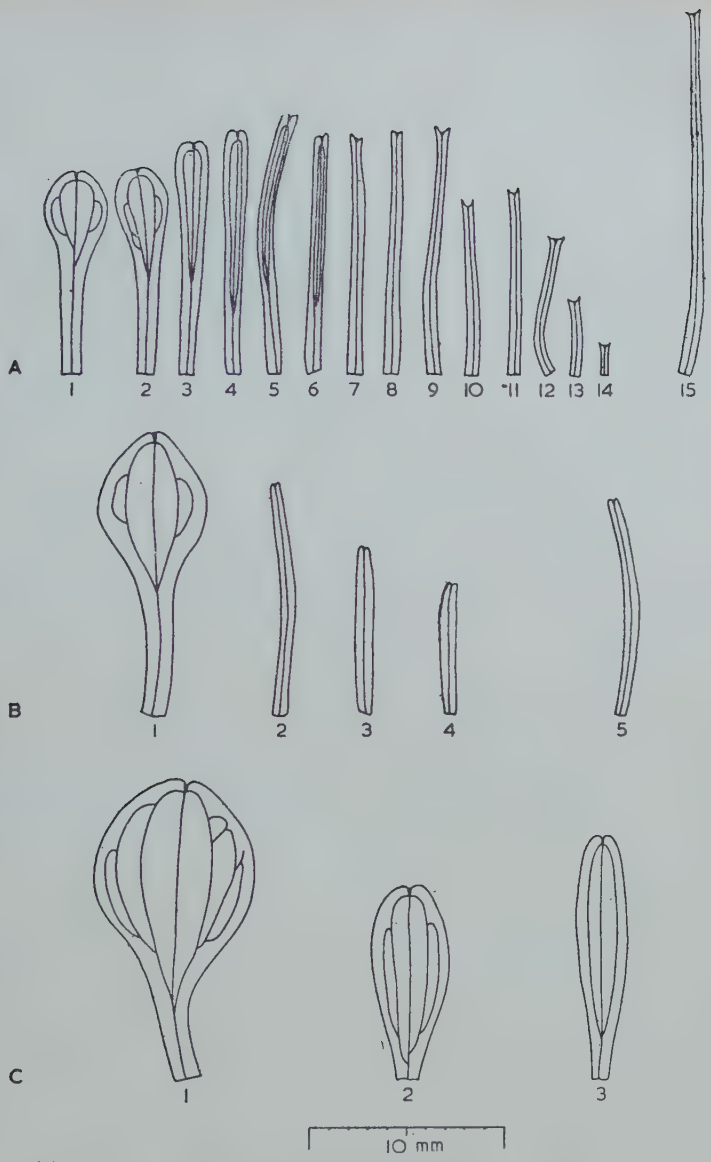
C. intermedia, *C. obtusangula*, and *C. stagnalis* all inhabit shallow, still water, the floating crowns of the plants often covering large areas of the surface. Under these conditions, all three species produce ovate, multi-veined leaves from the floating crown. Where axillary shoots develop under water the leaf form does not change in *C. stagnalis*, but *C. obtusangula* and *C. intermedia* both produce linear single-veined leaves on submerged axillary shoots.

C. intermedia can also grow successfully in running water where the plant is usually submerged as in the river Rheidol, a swift river of north Cardiganshire where it is a dominant aquatic species. When submerged in running water, linear leaves are produced but the linear form is more accentuated than in still water and displays a characteristic pincer tip.

The work to be described consisted of transplanting ovate-leaved shoots of the three species from still water to running water in order to ascertain the leaf form which would be developed in *C. stagnalis* and *C. obtusangula* as compared with that in *C. intermedia*.

Methods. Material was obtained from a drainage ditch at Tanybwlch, near Aberystwyth, where the three species occurred together. Shoots of about 15 cm. in length were taken, all the leaves of which were of the ovate type. Four groups, each of 12 shoots of each species, were 'planted' in the Rheidol about 2 ft. from the bank at a point some $2\frac{1}{2}$ miles from the mouth. In this situation *C. intermedia* occurs naturally. The bases of the shoots were weighed down with stones after being inserted in the small amount of silt to be found on the river bed. Depth of water at the time of planting was about 15 in., representing a low level. *C. intermedia* and *C. obtusangula* transplants were made on July 14, 1951, and *C. stagnalis* transplants on August 15, 1951.

Results. Up to the time the transplants were last noted (October 15, 1951) all the plants were growing well. The shoots of the *C. stagnalis* showed an increase in length of some 7 to 10 cm. and the leaves were still green and healthy. The *C. intermedia* and *C. obtusangula* shoots showed increases of some 30 cm. in length and stones had to be placed on the lower parts of the shoots to prevent the crowns reaching the surface. By April 1952 the *C. obtusangula* and *C. stagnalis* had disappeared. In the positions where the *C. intermedia* had been introduced there were several clumps of linear leaved plants, but it was impossible to say whether these were the original plants or whether a natural colonization had occurred. The state of the leaves of *C. intermedia* and *C. obtusangula* after 1 month's submergence is shown in Text-fig. 1A (1–14) and 1B (1–4), that of *C. stagnalis* after 2 months' submergence in Text-fig. 1C (1–3). After 3 months the leaves produced by *C. intermedia* had become very attenuated, while very little further elongation was shown by the new leaves of *C. obtusangula* (Text-fig. 1A, 15, and 1B, 5).



TEXT-FIG. 1. Ovate-leaved shoots submerged in running water.

A. *C. intermedia.* 1–14. (Submerged 1 month.) Leaves from successive nodes. 1 is an original ovate leaf. 14 is a young leaf from inner crown. 15. (Submerged 3 months.) Crown leaf.

B. *C. obtusangula.* 1–4. (Submerged 1 month.)
1 is an original ovate leaf.
2–4 are successive leaves from crown of axillary shoot. (4 is the youngest leaf.) 2 was the most attenuated linear leaf produced after 1 month, the crown leaves of the main axis being more ovate in shape.
5. (Submerged 3 months.)
Crown leaf (main axis).

C. *C. stagnalis.* 1–3. (Submerged 2 months.)
1 is an original ovate leaf.
2 is a representative crown leaf of main axis. 3 is the most linear leaf produced on any of the shoots (main axis).

(*b*) *The juvenile leaves of C. intermedia, C. obtusangula, and C. stagnalis*

In addition to the extremes of form shown by the leaves of the mature plants, information was also required as to the form of the seedling leaves. Though there is little variation in the adult leaves of *C. stagnalis* it was thought that the seedling leaves might show a different form from those of the mature plant. Again, the form of the seedling leaves was of importance in view of the possible light thrown on the phylogeny of the genus.

Methods. Seedlings were grown as follows. Mature fruits were placed in water in Petri dishes. Whereas the fruits float, when these split open and liberate the seeds, the latter sink immediately. A gelatinous covering on the surface of the seeds results in their adhering firmly to the bottom of the dish (a device of some value in the natural environment). There appears to be no dormant period. The seedlings shown in Pl. II, A, were typical specimens of a large number which were grown in this way, the parent plants being ovate-leaved plants from the same source as provided material for the transplants. Seedlings were fixed in formalin-acetic-alcohol which rendered the leaves sufficiently transparent for details of venation to be visible in transmitted light photographs.

Discussion of results (*Experiments a and b*)

The ovate-crowned *C. intermedia* from still water is capable of producing the extreme linear leaf with the pincer tip after a month's submergence in running water. This change is accomplished within a space of nine nodes. It is evident, too, from the intermediate types of leaf produced that the primordium remains plastic at an advanced stage in development. In *C. obtusangula*, the same period (and up to 3 months) of submergence does not produce a claw tip to the leaf. However, under conditions of submergence in running water the leaf approximates much more closely to a linear form than the leaf produced under conditions of still water. This latter resembles the juvenile leaves of the seedling (Pl. II, A). *C. stagnalis*, when submerged in running water, produces a somewhat elongated leaf. It would seem, at least for such short periods as 2 months, that the plant can tolerate submergence as such. The disappearance of the plant during the winter months when the speed of the current rises may be due to the resistance of the ovate leaves. *C. stagnalis* is present in abundance in the ox-bows of the lower Rheidol and would be likely to colonize the main river if conditions were suitable (*C. obtusangula* is absent from both ox-bows and main river).

In *C. intermedia* the first leaves produced after the cotyledons are linear. In *C. obtusangula* these leaves are linear-ovate, while in *C. stagnalis* they are more or less linear. In all cases these juvenile leaves are 1-veined. This is of particular interest in the case of *C. stagnalis*, since the leaves found on mature shoots after 2 months' submergence were still multi-veined. It seems curious that the juvenile leaves of *C. obtusangula* should be rather more ovate

than those of *C. stagnalis* while in the adult leaves the reverse should be the case.

From a consideration of the seedlings of the three species it is suggested that the primitive form of the leaf in the genus was linear.

Some Features accompanying the Submergence of Floating Axes of *Callitriche intermedia*

As has been seen, the submergence of floating axes results in the change in form from ovate to linear of those leaves which are at a sufficiently early stage in their development to be capable of the change. Another feature is that the axes on submergence show a high growth rate for a time. The falling off in the growth rate appeared to be related to the appearance of the linear leaves. The growth curves of such shoots were therefore studied in view of the possibility that the change in leaf form might be reflected in a change in the growth curve.

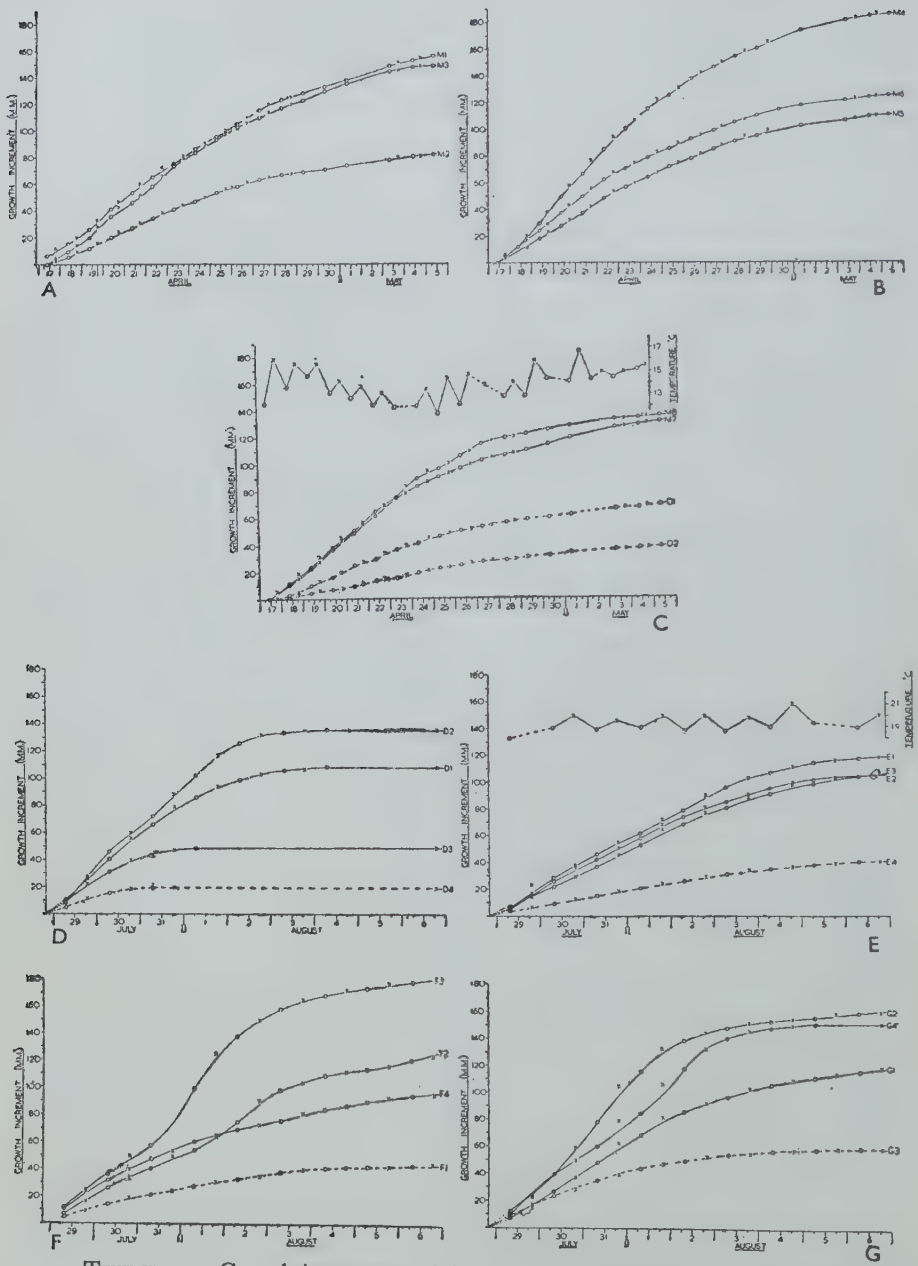
(*a*) *Growth of ovate-crowned shoots after submergence*

Series 1

Methods. The growth of the axes was measured by means of a vertically travelling microscope, the shoots being confined in lengths of glass tubing (of inside diameter 7 mm.) arranged vertically. This arrangement maintained the growth of the shoots in a straight line. Tanks of $5\frac{1}{2}$ litres capacity were used. In view of the short duration of the projected experiment tap-water was used with no added nutrient (Aberystwyth water is derived from a small corrie lake which is itself the source of the Rheidol). Plant material consisted of 8 ovate-crowned shoots and 2 linear-crowned shoots, the latter being measured for purposes of comparison. Plants were obtained from an ox-bow of the lower Rheidol, the linear-crowned shoots being axillary branches of the ovate-crowned shoots. Lengths of the latter shoots were such as to include 5 internodes and the former 4–5 internodes, an internode being recorded where it exceeded 1 mm. Shoot lengths were measured morning and evening, beginning on April 17, 1951. Lengths of individual internodes were also measured, although the data has not been used in the present work.

Results. Results are expressed graphically in Text-fig. 2 (A, B, C). Increments of growth are expressed directly rather than as a percentage of the original length since the increments appeared to bear no relation to initial shoot lengths. Appendix Table I indicates the time of appearance of linear leaves in the submerged crowns.

Discussion. No marked breaks are seen in any of the curves which might indicate physiological changes associated with the change in leaf form. Growth was uniform for several days at the beginning of the experiment and then a slow falling off in the rate occurred. This was the case with the linear and ovate shoots and might be due to the conditions of the experiment, inasmuch as the solutions were not aerated nor were nutrient salts available to the plants. One curious feature is noticeable, especially with the ovate-crowned shoots in the early, rapid growth phase. In general, day growth is



TEXT-FIG. 2. Growth increments of *C. intermedia* shoots when submerged.

Solid lines—Ovate-crowned shoots.
Interrupted lines—Linear-crowned shoots.
o—morning record.
x—evening record.
} (*All times in British Summer Time.*)

Series 1. A, B. C. Series 2. D, E, F, G.
(For explanation see text.)

greater than night growth. This is in contradiction to the usual distribution of growth over the day and night periods. It may be that this unusual periodicity of growth is peculiar to water plants or possibly to all plants growing under conditions of low light intensity. Light intensity during the daylight hours may be sufficient to provide adequate growth during the day but leaving insufficient nutritional reserves for higher night growth.

With regard to the order of appearance of linear leaves a point of some interest emerges. M2, the shoot on which ovate-linear leaves were first recorded (April 23), showed the least growth of all ovate-crowned shoots. This does not appear to be coincidental since M2, M5, M6, and M7, the four shoots which produced linear leaves by May 2, also had the least growth. By this time the remaining shoots had ovate-linear crowns except M3 (ovate/linear to linear).

At this stage of the work insufficient was known concerning the source of energy for the elongation following submergence. It might be that the energy was present in the form of reserve carbohydrates, which the shoot must consume owing to the possible inability to photosynthesize of submerged ovate leaves. On the other hand, these leaves might be active photosynthetically under water, in which case energy for the continued elongation was provided directly. In the former event it would seem that a low initial nutritional reserve would account for the slower growth of shoots which produce linear leaves first. In this case, the production of the linear leaves is associated with nutrition, as has been maintained for many other water plants. If, however, light was the source of the energy resulting in rapid elongation and consequent delay in formation of linear leaves, then depriving plants of light might hasten the formation of linear leaves.

In an attempt to solve some of these questions, a series of experiments was devised based on the principle of sucrose feeding of plants by Juhren and Went (1949). Part of the experiments entailed growing ovate-crowned shoots in the dark submerged in a solution containing nutrient salts with sucrose in order to examine the purely nutritive effect of light on shoot growth. Sulphanilamide was added with the object of preventing the growth of microorganisms. This latter precaution was only partly successful and the experiments had to be abandoned. Although this method failed and no means were available to supply carbohydrates artificially, there remained some problems which could still be investigated by normal culture methods. One problem was the effect of complete darkness on the one hand and continuous light on the other on the change from ovate to linear leaves. Also needing investigation was the possibility of a temperature effect operating in the change (possibly of the nature of thermoperiodism). The experiments described in Series 2 were designed to investigate these questions.

Series 2

Methods. Four groups of plants were used in this series, one in each tank. Group D was kept in the dark except for 15 minutes in the morning and

15 minutes in the evening (for measuring and aerating) and at a constant temperature of 25° C. Group E was subject to the normally fluctuating diurnal temperature and to the normal day/night illumination. Group F received normal day/night illumination, but the temperature was kept constant at 25° C. Group G received normal daylight, but during the night artificial light was supplied. Temperature was kept constant at 25° C. Three ovate-crowned shoots and 1 linear-crowned shoot were grown in tubes in the usual manner in each tank. In addition, 2 ovate-crowned shoots and 2 linear-crowned shoots were grown in each tank. These latter shoots were not confined in tubes but merely tied loosely to a glass rod and held submerged in that way. The 'free' shoots were included to check any effect which the confining action of the tubes might have.

The culture solution was based on Robbins's data (1941) for the main nutrients, while the micronutrients were introduced in a combination based on Haas and Reed's A–Z solution (Curtis and Clark, 1950). Details of the nutrient solutions are given in Appendix Table III. For use in the present work the nutrient solutions were diluted to $\frac{1}{10}$th the stated concentration (Pearsall and Hanby, 1925). The tanks were heated with 100-watt 'Angel' A1 heaters placed one at the base of each tank, the temperature being controlled with 'Angel' submersible thermostats, placed each near the surface of the water. Sufficient stirring was effected by convection currents to maintain the temperature in each tank at $25 \pm 0{\cdot}75$° C. Each tank was covered with a loose cap of cellophane. Artificial light in the case of the group G plants was supplied by 2 gas-filled 60-watt bulbs, each 1 foot from the shoots. The lamps were switched on half an hour before dusk and switched off 2 hours after dawn.

Group D plants were maintained in darkness by placing the tank in a large box with a light-trapped sliding door. All tanks were aerated for 15 minutes in the morning and 15 minutes in the evening. After each aeration the water in the tubes was replaced with aerated water using a syringe.

Ovate-crowned shoots were obtained from an ox-bow of the lower Rheidol, while linear-crowned shoots were taken from the main river. Each shoot possessed 5 internodes, each exceeding 1 mm. Each shoot bore either flowers or fruits. (In the previous experiment no record had been made as to whether the shoots bore flowers or fruits or were vegetative. In case this factor had some relation to the growth behaviour of the shoot, in the present series uniform material was used.) Shoots were measured morning and night. The timing of the measurements was such that 12 hours elapsed between records. pH of the solutions was checked at intervals throughout the experiment (colorimetrically). No variation was observed from an initial value of 6·8.

Results. Text-fig. 2 (D–G) shows the growth of the shoots expressed graphically. Appendix Table II shows the condition of crown leaves during the experiment.

Discussion (*ovate-crowned shoots*). While initial growth was rapid in group D shoots, growth ceased after a week. No linear leaves were formed on the

ovate-crowned shoots, but marked elongation of the axis occurred (D_1 and D_2). The shoots were devoid of chlorophyll in the upper portions, the upper internodes were elongated, and the crown leaves small. It is interesting to note that these typical features of etiolation were produced in plants exposed to as much as 30 minutes of light daily.

The change in leaf form is not, then, primarily associated either with the rapid elongation or with starving the plants through lack of light. The higher day growth in comparison with night growth was again seen, in general, in the early stages of elongation. Since temperatures were constant and the only light falling on the plants was 15 minutes at 7 a.m. and 15 minutes at 7 p.m., it appears that this fluctuating growth rate was a consequence of an inherent periodicity in the plant.

The group E shoots showed a similarity in growth rates and the growth rate was steady for about the first week. The higher day growth was again evident in this early period. Again, the first shoot to show linear leaves was E_3, which showed the least growth during the greater part of the experiment.

Group F shoots present a curious feature. F_2 was the only shoot which produced linear leaves (and also showed the lowest initial growth rate). The crowns of F_3 and F_4, which bore only ovate leaves, resembled closely the crowns of group D shoots. It would seem that the temperature increase of some 5 to 6° C. caused the difference in reaction between group E and group F shoots. (The temperature in E fluctuated, but the amplitude of the variations seemed scarcely large enough for a thermoperiodic mechanism to be involved.) Light intensity appears to be an important factor which also is closely linked with temperature. Under the comparatively low light intensities prevailing at the shoots (reduction in light intensity being caused by the tank and tube walls as well as the water) the change from ovate to linear leaves requires a relatively low temperature. When the temperature is raised, the mechanism effecting the leaf form change is upset. Under these circumstances the plants assume the characteristics of etiolation. The group F plants were probably at the threshold values of temperature and light intensity, accounting for the fact that one of the three shoots actually produced linear leaves. It is significant that the free plants of group F both produced linear leaves, the light intensity being higher at the free plants than at those enclosed in glass tubes.

Rapid growth was shown by all group G shoots initially, the day growth rate being markedly higher than the night growth during this initial phase (apart from the first one or two days). No priority in the formation of linear leaves was recorded. It is evident that the additional light period hastened the onset of the linear leaved condition. It is noteworthy that the initial growth rates of group D and group G shoots were very much alike. In the former, the high growth rate was associated with etiolation and crowns remained ovate. In the latter, no etiolation occurred and crowns soon produced linear leaves. It appears that under conditions of high light intensity, a rapid growth rate does not prevent the formation of linear leaves.

Linear-crowned shoots. Linear-crowned shoots remained linear-crowned for the duration of the experiments. In the case of the group D and group F experiments this result is of some interest. In these groups the majority of the ovate-crowned shoots failed to form linear leaves. Considering that the linear crowns survived unchanged, it is evident that the conditions of the experiment (D and F) merely inhibit linear leaf formation and do not result in ovate leaf formation.

(*b*) *Growth of ovate-crowned shoots of C. intermedia after submergence, with especial reference to leaf movements*

Methods. Two shoots bearing ovate leaves at the crown and along the axis were used, the source of the plants being an ox-bow of the lower Rheidol. The shoots were attached to glass rods submerged in tap-water in a small tank, the sides of which were of plate glass (cleaned photographic half-plates). The shoots were arranged close to that face of the tank nearest the camera, which was mounted on a tripod with a centre pillar which was used to compensate for growth of the shoot axes.

Illumination was supplied by a 100-watt bulb and by a No. 1 'Photoflood' bulb, each 30 cm. from the plants and directed at 45° to the camera/object axis. The tank was placed below a window.

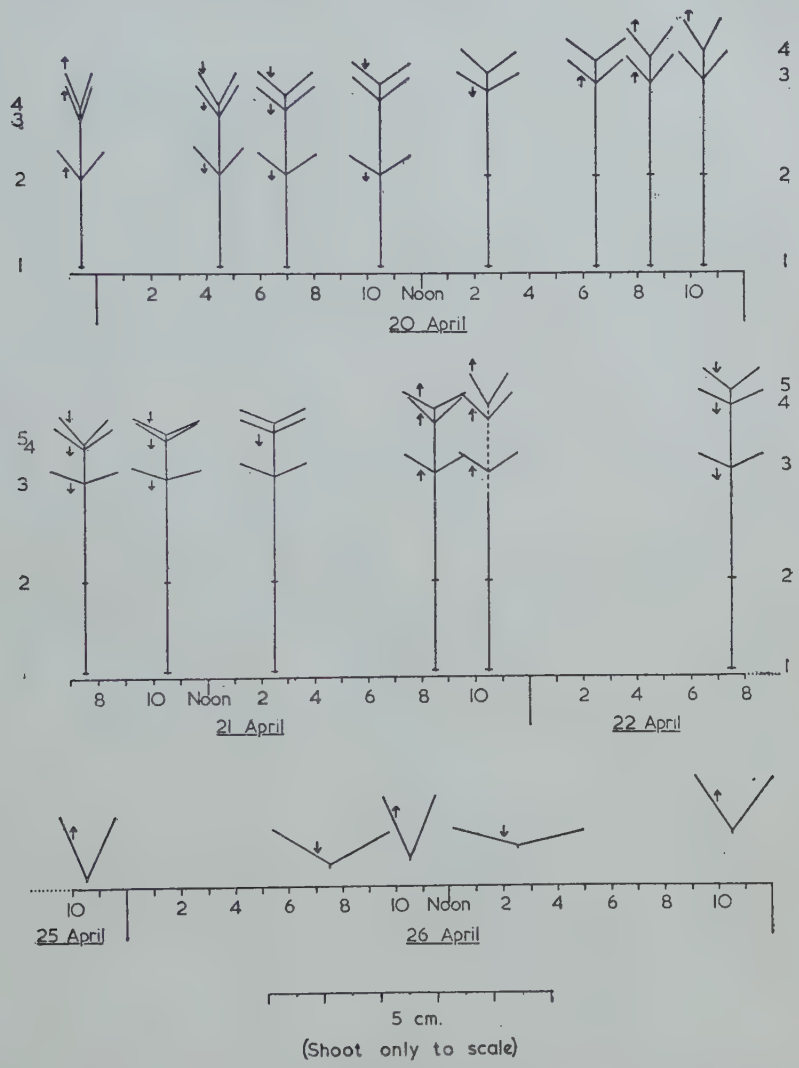
The experiment was started at 5 p.m. on April 17, 1952, and photographs were taken at approximately hourly intervals daily, from about 7 a.m. to 10.30 p.m. with earlier and later readings on some days (all times quoted have been adjusted to British Summer Time). From noon on April 20, with each photographic exposure, the temperature of the surface water was taken, as was a light reading (with a Weston Master Photoelectric Meter) of the daylight reflected from a white card held at the top of the tank (Appendix Table IV). In addition, from noon on April 20 a record was kept of the inclination (resulting from nutation) of each shoot towards or away from the camera. This was necessary since, if any measurements were to be taken from the photographs, it was important that only those records be used where the shoot was vertical to the optical axis of the camera.

In the following description reference is made mainly to one of the shoots, which produced a straight axis suitable for measurement. This shoot proved very convenient also for the study of leaf movements since a departure from the normal decussate arrangement of leaves resulted in several successive leaf pairs being orientated at right angles to the camera axis.

When this series of photographs had been completed some additional records were made. The water in the tank was lowered to a point where the tips of some of the crown leaves of each shoot were touching the surface of the water. A number of photographs were taken of the subsequent course of events, both from the side and, later, from above.

Results. From photographic prints of some of the negatives, two figures were constructed. Text-fig. 3 shows leaf movements and internode elongation in the early period after submergence of the shoot. The figure also

indicates the leaf movements at a later stage of submergence, when linear leaves were evident at the submerged crown. Text-fig. 4 illustrates the growth of an internode (3–4 of Text-fig. 3), constructed from measurements taken



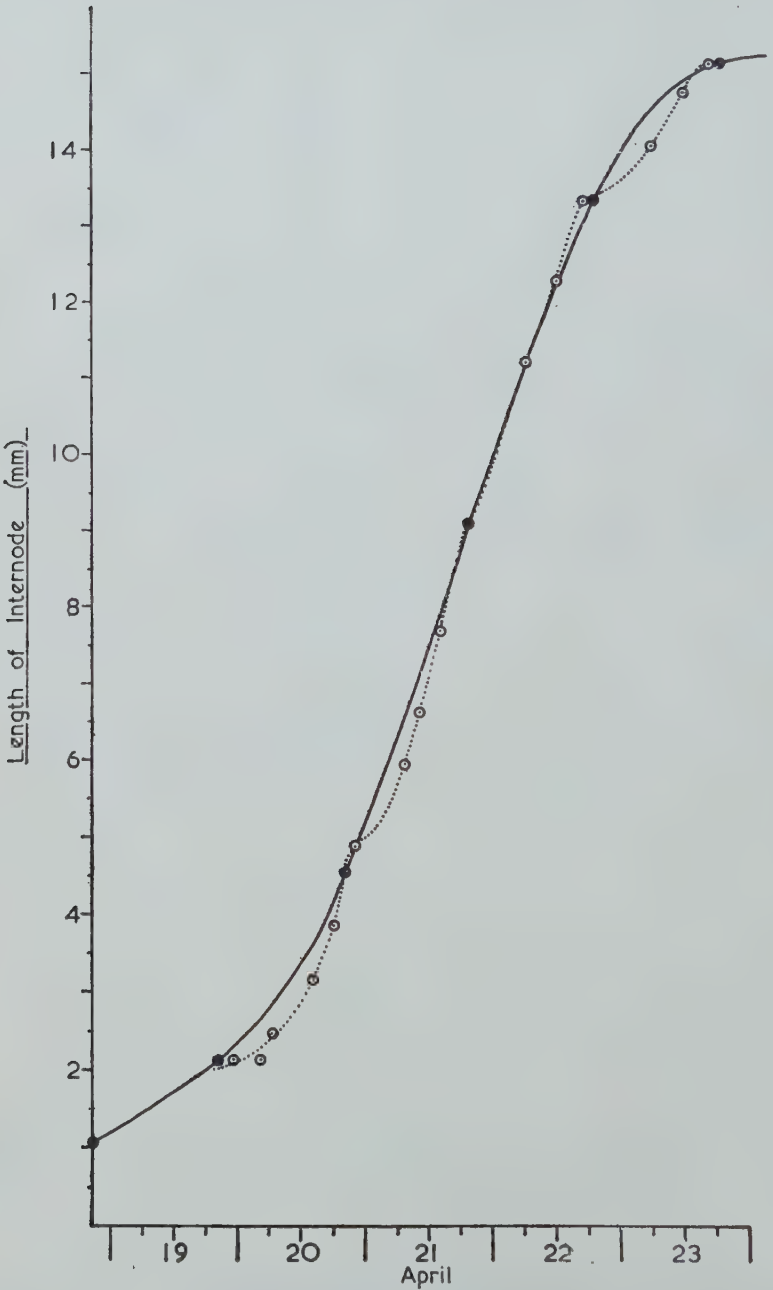
TEXT-FIG. 3. Leaf movements following submergence of an ovate-crowned shoot of *C. intermedia*.

Arrows refer to direction of leaf movement with respect to previous record.

(*All times in British Summer Time.*)

from a specially enlarged series of prints. A selection of photographs is shown in Pl. II, B, C, D.

There was no appreciable leaf movement on the day of submergence. Subsequently leaf movements were seen, the degree of movement varying with



TEXT-FIG. 4. Growth of a single internode of an ovate-crowned shoot of *C. intermedia* when submerged.

Solid Line—Plot of lengths at 8.30 p.m. (B.S.T.)
Interrupted line—Plot of lengths throughout the day.

the position of the leaf on the axis. The crown leaves were capable of the greatest range of movement and, where internode elongation was evident, the range of movement of the leaves thus separated from the crown was decreased. After a certain time, the leaves were incapable of further movement. Leaf pair 1 had not moved appreciably since beginning the record. Leaf pair 2 showed no movement after 10.30 a.m. on April 20, while leaf pair 3 showed no further movement after 7.30 a.m. on April 22.

During the early period of submergence the leaves were at their most 'open' position at about midday and were fully closed probably towards midnight. It may be seen from Text-fig. 3 and Pl. II, B, that the leaves had opened appreciably by 4.30 a.m. on April 20 (when it was still dark) from their position at 11.30 p.m. on April 19. First light was at 5 a.m. on April 20. During the later period of submergence, when linear leaves were evident at the crown, a change in the rhythm of leaf movement was observed. Superimposed on the previously recorded movements there was a closure towards midday (Text-fig. 3, Pl. II).

The general growth of internode 3/4 followed the 'S' form, showing a low initial rate of elongation over the first day, then a marked acceleration over the next 3 days, while in the final day the rate slowed down again. An interesting feature is that the daily growth curve also followed the 'S' form, slow growth being characteristic of the night and early morning and a rapid growth occurring later in the morning and during the afternoon. The results show in detailed form what was evident from the measurements of the growth of submerged shoots in the previous section, namely that the day growth proceeds at a higher rate than the night growth.

From a comparison of the growth curves with the leaf movements it seems that the rapid growth is associated with the open condition, while slow growth occurs when the leaves are closed. The first recorded length of internode 3/4 is at 8.30 p.m. on April 18. At this time the leaves of node 2 were still performing movements. When the leaves at node 2 had attained their final position at 10.30 a.m. on April 20 it may be significant that internode 3/4 was entering on its phase of most rapid elongation. The leaves at node 3 attained their final position at 7.30 a.m. on April 22, towards the end of the phase of rapid elongation. At this point the internode above was probably entering on its phase of accelerating elongation rate. The elongation of internode 3/4 was completed some time during the night of 23–24 April. The leaves at node 4 attained their final position by 7.30 a.m. on April 24.

Discussion. Bünning (1948) asserts that the leaf movements of plants are symptomatic of an endogenous periodicity which is the basis of photoperiodicity. The possibility of there being a photoperiodic mechanism involved in the change in leaf form has not been pursued in the present investigation. Such a possibility would have to take into account the fact that the ovate floating crown leaves do not show leaf movements, that there are no leaf movements on the day of submergence, and that a certain type of movement then occurs, which alters when linear leaves are visible at the crown.

Yin (1941) attributes the leaf movements of *Carica papaya* to differential distribution of auxin in the petiole. He also postulates an autonomous periodicity in auxin production. The close relation between leaf movements and internode elongation in *C. intermedia* would suggest that here also the leaf movements are associated with auxin formation. It is likely that the auxin is formed in those leaves which perform movements and is translocated out of the leaves at the time when these are open, resulting in rapid elongation of the internodes. The end of internode elongation is associated with the end of leaf movements of the upper node of the internode concerned, the leaves of the lower node having ceased auxin production (and movement) some time previously. If these general assumptions are correct, then the growth rate would be higher in the upper parts of the internode than in the lower (and growth would cease first in the lower part of the internode). Such a distribution of growth within the internode has, indeed, been reported in other plants (Bonner and Galston, 1952).

While there seems to be a general relationship at times between light intensity and leaf movement and between temperature and leaf movement, it is not considered that these factors are responsible for the leaf movements. The temperature fluctuations during the leaf movements were in many cases slight. The leaves also began to open before first light. In addition there appears to be a strong correlation between internode elongation and leaf movement and it has been seen in the preceding section that the diurnal fluctuation in total growth rate of the shoot is unaffected by light or temperature. During the later period of submergence a change in the type of leaf movement occurs which is related to the appearance of linear leaves at the crown. It might be argued that high light intensity caused midday closure on April 26. An autonomous rhythm is, however, the most likely explanation in view of the usual night closure and also for the appearance of the two shoots at 8.30 a.m. on April 26. Here the movements are slightly 'out of phase', the right-hand crown beginning to close before the left hand (the light intensity at this time being still low).

The lowering of the level of water in the tank had a marked effect on the leaf movements. At 10.30 p.m. on April 27 no leaf had broken through the surface although, if the crowns had been submerged, the leaves would have been placed at a more acute angle than they had in fact assumed. Surface-tension forces prevented the relatively delicate leaves from breaking through the surface, although some purely physiological mechanism brought into action by the arrival of the leaves at the surface cannot be discounted as interrupting the normal course of the leaf movements. With continued growth of the shoot the crown approached the surface. This resulted in the crown leaves assuming a still more horizontal position. Photographs taken vertically downwards on to the crown breaking the surface show that most of the inner crown of the plant (where ovate leaves were forming) was submerged. Virtually only the distal parts of the older crown leaves actually broke surface so that the upper surface of the leaf was exposed to the air. In any case,

even with fully developed floating crowns, it is very unlikely that exposure to dry air of the inner crown is the cause of formation of ovate leaves since the very young leaves are enclosed within the older leaves and are certainly surrounded by layers of humid air if not of water. The older leaves are the first to reach the surface, being longer than the younger leaves, and it is this fact which is probably responsible for the inception of the ovate-leaved condition. The stimulus may be dryness of the air following emergence, transmission of the stimulus to the apex being in the form of auxin translocation. However, it should not be ignored that one physical result of the leaves coming to the surface is their forced adoption of the horizontal position (the floating leaves of the crown perform no movements apart from the adoption of a horizontal position on attaining a certain length). This may, in itself, initiate the mechanism for change in leaf form.

Summary

1. Transplants of ovate-leaved shoots of *C. intermedia*, *C. obtusangula*, and *C. stagnalis* to conditions of submergence in running water show that in *C. intermedia* the extreme linear leaves are formed very readily. *C. obtusangula* shows a more linear type of leaf than is found under still water conditions while *C. stagnalis* shows the least departure from the ovate leaf form.

2. Juvenile leaves of the three species approximate to the linear, a curious feature being that the juvenile leaves of *C. stagnalis* are more linear than those of *C. obtusangula*, although in the adult leaves the reverse is the case. It is tentatively suggested that the primitive form of leaf in the genus is linear.

3. On submergence of the ovate-crowned shoot of *C. intermedia* there is an initial period in which a high growth rate is maintained. The rate then falls off, possibly as a result of the utilization of food reserves. During the initial rapid growth phase, day growth is higher than at night. This is attributed to an inherent periodicity in the plant and not to the influence of external factors such as light and temperature.

4. Light is essential for the change from ovate leaves to linear leaves in *C. intermedia*, but the inhibition of the change occurs even if the shoots are subjected to 30 minutes of light daily. Under normal daylight conditions an increase in temperature from 20 to 25° C. is sufficient to prevent the formation of linear leaves in 2 out of 3 shoots. It is suggested that this indicates a delicate balance between temperature and light. If the temperature is high relative to the prevailing light, linear leaves fail to form. Low light intensity does not induce the change from linear to ovate leaves.

5. On submergence of ovate-crowned shoots of *C. intermedia*, linear leaves are formed first on shoots which exhibit least growth. A high growth rate under conditions of low light intensity may be indicative of incipient etiolation with an accompanying retardation of the change in leaf form. Full daylight, supplemented by artificial light at night, results in early formation of linear leaves in submerged ovate-crowned shoots which are maintained at 25° C.

The high growth rate under these conditions of ample light is not associated with any of the features of etiolation.

6. The rate of elongation of a linear-crowned shoot of *C. intermedia* when submerged is appreciably lower than that of a submerged ovate-crowned shoot.

7. On submerging an ovate-crowned shoot of *C. intermedia*, a short period elapses during which the leaves show no movements. Subsequently diurnal leaf movements occur, probably due to an endogenous periodicity. Leaves open during the morning and afternoon and close during the evening and night. When the linear leaf movements are prominent at the crown, the periodicity of the leaf movements is complicated by the additional closure of the leaves at midday.

8. The elongation rate of an internode of the submerged shoot of *C. intermedia* follows the normal 'S' type over a period of some 5 days. The daily growth rate is also of the 'S' type, the increased growth coinciding with the open-leaved period. It is suggested that the open-leaved period conforms to the passage out of auxin resulting in the rapid elongation of the internodes. The end of the internode elongation is associated with stationary leaves at the nodes immediately above and below.

9. When a linear crown approaches the surface, inception of ovate leaves at the apex follows from the appearance at the surface of older crown leaves. It is suggested that the stimulus is probably transmitted from these older leaves to the developing primordia in the form of an auxin, though it is questionable whether exposure to the air of the leaves or the maintenance of the leaves in the horizontal position occasions the primary stimulus.

10. No leaf movements corresponding to those observed in submerged crowns were detected in the floating crowns of *C. intermedia*.

Acknowledgements

I wish to thank Professor Lily Newton for reading through the script of this paper. Part of the expenses involved in undertaking this work was met by the Ministry of Agriculture and Fisheries to whom my thanks are due.

Appendix Table I

Series 1. Form of Crown Leaves (Originally Ovate)

Date.	Shoot.	Form of crown leaves.
April 23	M2	Ovate/linear
April 25	M1	Ovate
	M2	Linear
	M3	Ovate/linear
	M4	Ovate
	M5	Ovate/linear
	M6	Linear
	M7	Ovate/linear
	M8	Ovate to ovate/linear
May 2	M1	Ovate/linear
	M2	Linear
	M3	Ovate/linear to linear
	M4	Ovate/linear
	M5	Linear
	M6	Linear
	M7	Linear
	M8	Ovate/linear

Appendix Table II

Series 2. Form of Crown Leaves (Originally Ovate)

Date.	Shoot (enclosed).	Form of crown leaves.	Shoot (free).	Form of crown leaves.
August 1	D	Ovate	D	Ovate
	E	Ovate	E	Ovate/linear
	F	Ovate	F	Ovate/linear
	G	Linear	G	Ovate/linear
August 2	D	Ovate	D	Ovate
	E1	Ovate	E	Linear
	E2	Ovate		
	E3	Linear		
	F2	Linear	F	Linear
	F3	Ovate		
	F4	Ovate		
	G	Linear	G	Linear
August 3	D	Ovate	D	Ovate
	E1	Ovate/linear		
	E2	Ovate/linear		
	E3	Linear		
	F2	Linear		
	F3	Ovate		
	F4	Ovate		
August 5	D	Ovate	D	Ovate
	E	Linear		
	F2	Linear		
	F3	Ovate		
	F4	Ovate		

Appendix Table III

Series 2. Composition of Nutrient Solution

Main nutrients (solution A).

$Ca(NO_3)_2$	0·333	g./litre
$MgSO_4.7H_2O$	0·083	,,
KNO_3	0·083	,,
KCl.	0·042	,,
KH_2PO_4	0·083	,,

Micronutrients and iron (solution B).

H_3BO_3	0·6	g./litre
$MnCl_2.4H_2O$	0·4	,,
$ZnSO_4.7H_2O$	0·09	,,
$CuSO_4.5H_2O$	0·05	,,
$FeCl_3.6H_2O$	2·4	,,
$Na_2MoO_4.2H_2O$	0·3	,,

1 ml. of solution B was included in a litre of solution A and the whole was then diluted to 10 litres for use.

Appendix Table IV

Sequence Photographs of the Growth of a Submerged, Ovate-crowned Shoot—Temperature and Light Records

Date.	Time.*	Temperature of surface water (°C.).	Light intensity at water surface (candles/sq. ft.).†
April 20	2.30 p.m.	17	37
	6.30 ,,	17	13
	8.30 ,,	16·8	0
	10.30 ,,	16	0
April 21	7.30 a.m.	13·6	4
	10.30 ,,	13·4	25
	2.30 p.m.	14·4	13
	8.30 ,,	14·8	0
	10.30 ,,	14·3	0
April 22	7.30 a.m.	12·3	6·5
	1.30 p.m.	14·4	37
	6.30 ,,	14·8	22
	8.30 ,,	14·5	0
	10.30 ,,	14·4	0
April 23	7.30 a.m.	12·3	11
	1.30 p.m.	14	75
	6.30 ,,	14·4	13
	8.30 ,,	14·4	0
April 25	10.30 ,,	16	0
April 26	7.30 a.m.	13	37
	8.30 ,,	13	75
	10.30 ,,	16	800
	2.30 p.m.	18·7	50
	10.30 ,,	16·9	0
April 27	10.30 ,,	16·3	0
April 28	8.30 a.m.	13·8	20
	5.30 p.m.	15·7	25
	6.30 ,,	16	13
May 3	1.30 ,,	14·5	25

* Times adjusted to British Summer Time.

† Light reflected from a white card held at surface of water.

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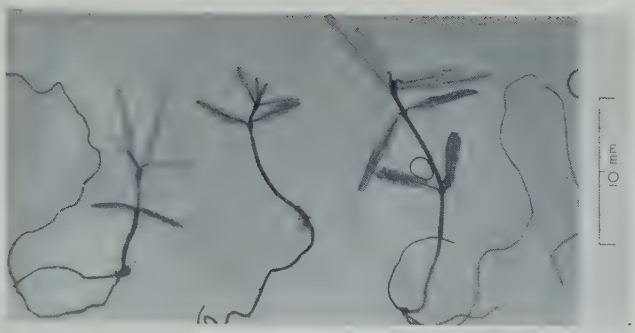
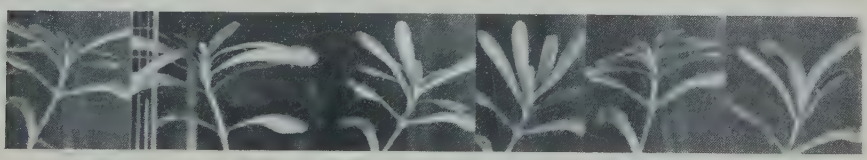


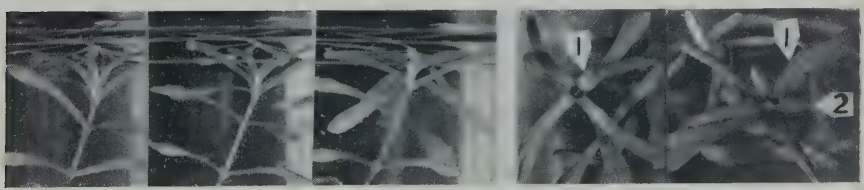
A

5 p.m. 17 April | 11.30 p.m. 17 April | 4.30 a.m. 20 April | 2.30 p.m. 20 April | 10.30 p.m. 20 April



7.30 a.m. 26 April | 8.30 a.m. 26 April | 10.30 a.m. 26 April | 2.30 p.m. 26 April | 10.30 p.m. 26 April

B



10.30 p.m. 27 April | 8.30 a.m. 28 April | 5.30 p.m. 28 April

C

6.30 p.m. 28 April | 1.30 p.m. 3 May

D

A. Seedlings of three species of *Callitriche*:
Left—C. intermedia at 30 days.
Centre—C. obtusangula at 30 days.
Right—C. stagnalis at 35 days.

B. (17–20 April). Leaf movements and axis elongation following submergence of an ovate-crowned shoot of *C. intermedia*.

B. (26 April). Leaf movements when linear leaves are present in the crown. (The left-hand shoot has been included at 8.30 a.m. to show that the two shoots may show leaf movements 'out of phase'.)

C. Linear crown in contact with the water surface.

D. The same crown viewed from above. 1 indicates identical leaves. Leaf pair 2 (3 May)

Experimental and Analytical Studies of Pteridophytes

XXVII. Investigations on *Marsilea*. 5. Cultural Conditions and Morphogenesis, with Special Reference to the Origin of Land and Water Forms

BY

A. ALLSOPP

(Department of Cryptogamic Botany, University of Manchester)

With Plates III and IV and six Figures in the Text

Abstract

Experiments are described in which sporelings of *Marsilea* were grown aseptically on media of similar nutrient value, but in which the osmotic concentration was changed by addition of mannitol, by alteration of the concentration of inorganic constituents, or by substitution of sucrose for glucose. Comparison is then made between the morphology of supported and submerged sporelings. Further experiments are described in which sporelings were transferred from high to low and from low to high sugar concentrations, from supported to submerged growth, and from glucose media to media containing mannitol.

The principal hypotheses which seek to account for the characteristic features of water plants are discussed in relation to the results of the above experiments. It is concluded that it is the water balance of the developing tissues, as determined by such factors as the osmotic pressure of liquid media, the relative humidity of the atmosphere, or more generally, the diffusion pressure deficit of the water of the environment, which leads to the appearance of the morphological features characteristic of land or water forms; nutritional conditions play only a subsidiary role.

[Annals of Botany, N.S., Vol. XIX, No. 74, April, 1955.]

Introduction

In earlier papers of the present series (Allsopp, 1952, 1953, 1953*a*, 1954) it was shown that the normal heteroblastic development of *Marsilea* is closely controlled by the nutrition of the developing sporeling. But there was no indication that the observed morphological differences between land and water forms of this plant have any direct connexion with either nutritional status or heteroblastic development. The available evidence pointed rather to the importance of osmotic phenomena, as influenced by the water relations of the environment. In the present paper an account is given of further experimental work on the factors responsible for the appearance of the distinctive features of the land and water forms of *Marsilea*. It will be shown that the features characteristic of naturally occurring land and water forms of *Marsilea* may also be produced by appropriate experimental treatments. Such experimentally produced forms will be referred to as 'land' or 'water' forms to distinguish them from the corresponding naturally occurring forms.

Materials and Methods

As in the earlier investigations the experimental work was carried out on *Marsilea drummondii* A. Br.

Aseptic cultures were obtained by the method described previously (Allsopp, 1952) and unless otherwise stated were grown on the same basic inorganic medium under the standard conditions of lighting and temperature.

Comparison of the effects of various treatments was always made between sporelings obtained by self-fertilization from one sporocarp. Other details are given in the accounts of the individual experiments.

Development in liquid media of different osmotic concentrations

Experiments on the effects of a range of concentrations of various sugars have already shown (Allsopp, 1953*a*, 1954) that sporelings grown in the higher sugar concentrations, e.g. 4 and 5 per cent. glucose, develop morphological and anatomical features characteristic of land forms of *Marsilea*, while in lower sugar concentrations, e.g. 1 and 2 per cent. glucose, features characteristic of water forms are found. The evidence presented led to the conclusion that these morphological differences are not a consequence of improved nutrition in the higher sugar concentrations but are a response to the higher osmotic pressure of the culture medium. This view has now been tested by growing sporelings in media designed to have the same nutrient value but different osmotic pressures.

(*a*) *Effects of addition of mannitol*

Mannitol, apart from its osmotic activity, appears to be physiologically inert in higher plants and since it penetrates into cells with great difficulty has already been used by a number of workers for raising the osmotic pressure of

their experimental solutions without affecting other physiological properties (Molliard, 1907; Thimann and Schneider, 1938; van Overbeek, 1942). It therefore seemed that, in the present work, addition of mannitol to the culture media would enable a distinction to be made between the effects of the nutrient content and the osmotic pressure of the medium. Two series of experiments of this kind have been carried out.

Series MD 8/3. Parallel sets of twelve sporelings per treatment were grown on the basic medium with addition of the following constituents: 1 per cent. glucose; 1 per cent. glucose+2 per cent. mannitol; 1 per cent. glucose+4 per cent. mannitol; 2 per cent. sucrose; 2 per cent. sucrose+2 per cent. mannitol; 2 per cent. sucrose+4 per cent. mannitol; 2 per cent. mannitol; and 4 per cent. mannitol.

Within a few days it became evident that mannitol is not utilized as a nutrient; the sporelings in both concentrations of the mannitol controls made no more growth than those in the basic inorganic medium alone, i.e. only a few simple leaves appeared.

It was also clear that mannitol is unused even in the presence of sugar. Thus the growth rate of sporelings in the sugar solutions to which 2 per cent. mannitol was added was appreciably less than in the corresponding solutions without mannitol. In general morphology, however, there was virtually no difference between the plants from the media with and without 2 per cent. mannitol. The heteroblastic leaf development was also unaffected, the adult quadrifid condition appearing at the same node, though later in time, in the two types of media. In the media containing 4 per cent. mannitol, growth was abnormal with fairly well-developed rhizomes but very stunted leaves, with only short petioles and practically no lamina.

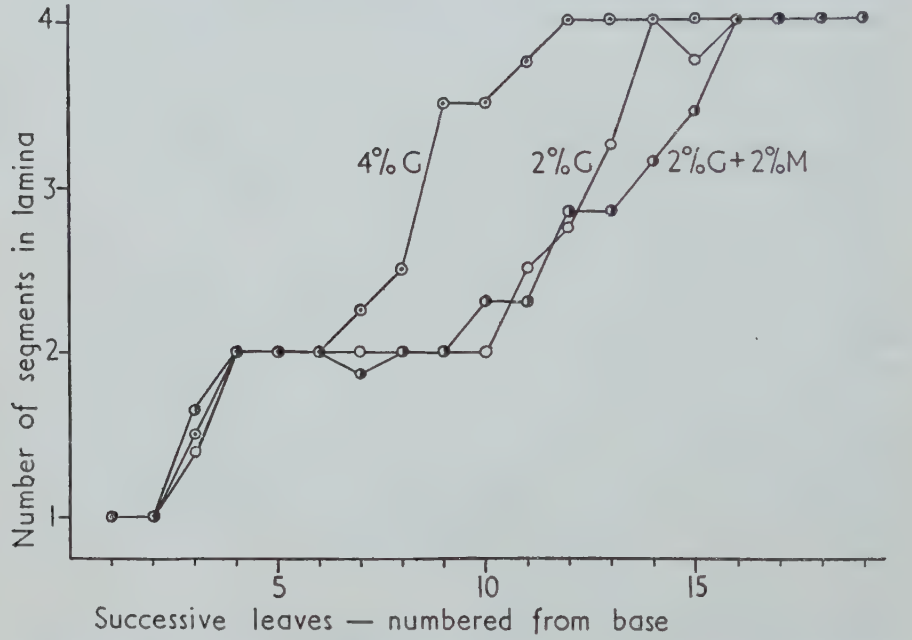
The results of this series indicated that in the next set of cultures a higher sugar concentration should be used.

Series MD 8/5. Parallel sets of ten sporelings per treatment were grown on the basic medium with addition of: 2 per cent. glucose; 4 per cent. glucose; 2 per cent. glucose+2 per cent. mannitol; 2 per cent. glucose+3 per cent. mannitol; 2 per cent. glucose+4 per cent. mannitol; and 4 per cent. sucrose+3 per cent. mannitol.

In this series of cultures the concentrations proved more suitable for the development of morphological differences. All sporelings in 2 per cent. glucose developed features characteristic of water forms, while the sporelings in 4 per cent. glucose had the features of land forms (Pl. III, Figs. 1 and 2). Of the eight surviving sporelings in 2 per cent. glucose+2 per cent. mannitol, five resembled typical land forms (Pl. III, Fig. 3) while the remaining three were stunted abnormal plants (Pl. III, Fig. 4) similar to those obtained in the higher mannitol concentration of Series 8/3. The five 'land' forms were stunted as compared with those in 4 per cent. glucose, but otherwise typical. In 2 per cent. glucose+3 per cent. mannitol the sporelings were even more stunted; three only had land-form characteristics, the other seven were similar to the abnormal plant illustrated in Pl. III, Fig. 4. In 2 per cent. glucose+4 per cent.

mannitol all plants were stunted and abnormal (Pl. III, Fig. 5). The sporelings from 4 per cent. sucrose+3 per cent. mannitol had the same stunted abnormal appearance (Pl. III, Fig. 6).

The results of both series of experiments support the previous conclusion (Allsopp, 1953*a*) that the effect of increasing sugar concentration on heteroblastic development is not of osmotic origin. In contrast with other morphogenetic effects produced, addition of mannitol to the culture medium had little or no effect on leaf segmentation. This is illustrated for Series MD 8/5

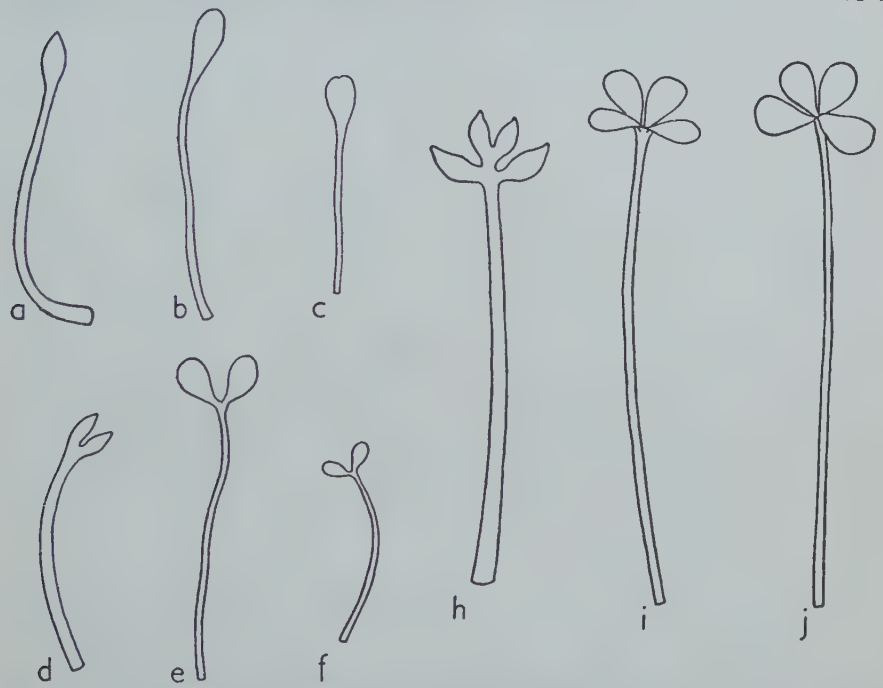


TEXT-FIG. 1. *M. Drummondii.* Effect of addition of mannitol on the segmentation of successive leaves. Mean values from eight parallel series of cultures. -O- 2% glucose; -⊙- 4% glucose; -◑- 2% glucose+2% mannitol.

in Text-fig. 1. Although the osmotic pressure of the medium containing 2 per cent. glucose+2 per cent. mannitol is approximately equal to that of the 4 per cent. glucose medium, there is a considerable lag in leaf segmentation, which is closely parallel with that in 2 per cent. glucose.

There can be little doubt, however, that the occurrence of land-form characteristics in sporelings grown on 2 per cent. glucose+2 or 3 per cent. mannitol is a response to the higher osmotic pressure of these solutions. The similarity in leaf form between a sporeling from 2 per cent. glucose+2 per cent. mannitol and one from 4 per cent. glucose is shown in Text-fig. 2. It will be seen that certain characteristic features of the 'land' leaf form, extend even to the bifid and the undivided juvenile leaves. The only marked difference is in the small size of the juvenile leaves from the solutions containing mannitol.

This stunting effect of mannitol was found to some extent at all concentrations, but increased with the concentration. Using other plant materials a number of workers have previously demonstrated that an increase in the osmotic concentration of the nutrient solution leads to a shortening of the axis. In the present experiments, however, it would seem that the reduced growth in media containing mannitol is also due to some interference with the absorption of sugar, since all parts of the plant are affected and there is some reduction even in relatively dilute solutions. Inadequate carbohydrate supply



Text-fig. 2. *M. Drummondii.* Effect of addition of mannitol on leaf morphology. (*a*), (*b*), (*c*) third leaf, (*d*), (*e*), (*f*) first bifid leaf, (*h*), (*i*), (*j*) first quadrifid leaf, from sporelings grown on 2% glucose, on 4% glucose, and on 2% glucose+2% mannitol respectively. All $\times 3\frac{1}{2}$.

may thus account for some of the abnormal features of the plants from media with high concentrations of mannitol.

Anatomical effects. In view of the marked similarity in morphological features between plants from 4 per cent. glucose and 2 per cent. glucose+2 per cent. mannitol, and the difference between these plants and those from 2 per cent. glucose, a comparison has been made of the anatomy of representative plants from the three media. An account of the anatomical features of plants from low and high sugar concentrations has already been given in a previous paper, in which sections taken from the sporelings shown in Pl. III, Figs. 1 and 2 of the present paper were used in illustration of the anatomy of typical plants from 2 and 4 per cent. glucose (Allsopp, 1954, Pls. 1, 2, and 3). It was shown that plants from the low sugar concentration have the anatomical

TEXT-FIG. 3. *M. Drummondii.* (*a*), (*c*), and (*e*) upper epidermis, (*b*), (*d*), and (*f*) lower epidermis, of the first quadrifid leaves of sporelings from 4% glucose, 2% glucose, and 2% glucose+2% mannitol respectively (leaves *i*, *h*, and *j* of Text-fig. 2). All ×235.

characteristics of water forms of *Marsilea* while plants from the higher sugar concentration have the anatomical features of land forms.

Examination of the anatomy of sporelings from 2 per cent. glucose+2 per

cent. mannitol has now shown that sporelings of the type illustrated in Pl. III, Fig. 3, are similar to those from 4 per cent. glucose, i.e. they have the anatomical characteristics of normal land forms. Text-fig. 3 demonstrates the similarity in stomatal distribution between the leaves of plants from 4 per cent. glucose and 2 per cent. glucose+2 per cent. mannitol. Both upper and lower epidermis have numerous stomata, in contrast with the condition in 2 per cent. glucose in which stomata are few in the upper epidermis and absent from the lower. The stomata from the 2 per cent. glucose+2 per cent. mannitol medium, however, differ from those from the 4 per cent. glucose solutions, in that the guard cells lack prominent cuticular bars and are not overgrown appreciably by the adjoining cells. It is clear from sections of the leaf that the internal structure is similar in 4 per cent. glucose and 2 per cent. glucose+2 per cent. mannitol (Pl. III, Fig. 7), but in the leaf from the medium containing mannitol there are only four layers in the mesophyll, as opposed to six in the leaf from 4 per cent. glucose, and the palisade tissue is not so clearly differentiated.

Other anatomical features of the plant from 2 per cent. glucose+2 per cent. mannitol are illustrated in Pl. III, Figs. 8, 9, 10, 11. Sections of root, petiole, and rhizome are similar to those from 4 per cent. glucose. There are fewer cells in the rhizome, but the general construction is of the same type. From the longitudinal section of the apex it is evident that this also is of the type found in higher sugar concentrations. The early expansion of the sub-apical region is even more marked than in the plants from high sugar concentrations, and the active meristematic region is correspondingly reduced.

It may be concluded from the above account that the 'land' forms from the 2 per cent. glucose+2 per cent. mannitol medium have basically the same type of anatomical construction as those from 4 per cent. glucose. The differences in the plants from the mannitol medium are of the kind that one would expect in plants receiving relatively inadequate carbohydrate supplies.

Examination has also been made of the anatomy of the abnormal sporelings shown in Pl. III, Figs. 4 and 5. It is of interest that the structure is basically of the type found in low sugar concentrations, but with differences in detail corresponding to the reduced dimensions.

(*b*) *Effects of changes in concentration of inorganic constituents*

In view of the pronounced morphogenetic effect of changes in the sugar concentration of the medium (Allsopp, 1953*a*), it seemed of interest to study the effect of changing the concentration of the Knop's solution supplied. Parallel sets of eight cultures were therefore grown on a medium containing 2 per cent. glucose, but with the Knop's solution at $\frac{1}{5}$, 1, 5, and 10× the normal concentration.

For the first few months of growth the concentration of the inorganic constituents was evidently not at a limiting level, since the amount of growth and the heteroblastic development of the leaves were closely similar at the $\frac{1}{5}$, 1, and 5× concentrations. In the 10× solution growth was markedly abnormal (Pl. IV, Fig. 12) and only three cultures survived.

The sporelings from $\frac{1}{5}$ and 1× Knop's solution were typical 'water' forms and have already been illustrated in a previous paper (Allsopp, 1953) in which reference was made to reversion to juvenile leaves in the $\frac{1}{5}$ concentration, probably following exhaustion of one or more essential mineral nutrients. In the 5× Knop's solution, however, half of the cultures developed some of the characteristics of land forms (Pl. IV, Fig. 13), although the shortening of the internodes was not so marked as in the higher sugar concentrations. It should be pointed out that the simultaneous occurrence of 'land' and 'water' forms is not surprising as the osmotic pressure of the culture medium (calculated as 3·99 atmospheres) is virtually identical with that of a normal 3 per cent. glucose medium (calculated O.P. 4·01 atmospheres) in which both 'land' and 'water' forms frequently occur. The calculated osmotic pressure of the normal 4 per cent. glucose medium, in which almost all cultures have characteristics of land forms, is 5·15 atmospheres.

(*c*) *Differential effects of glucose and sucrose*

In earlier work (Allsopp, 1953*a*) it was found that with glucose or fructose, 7·5 per cent. was the highest concentration permitting growth of *Marsilea* sporelings, while with sucrose this limit was attained only at a concentration of 15·0 per cent. This greater tolerance towards sucrose was attributed to the lower osmotic pressure of the sucrose solutions. In view of the importance now attached to the osmotic pressure of the medium as a factor in morphogenesis, it was considered desirable to confirm the previous result that sporelings with the characteristics of land forms were obtained at a lower concentration of glucose than of sucrose.

Embryos from the same sporocarp, 8 per cent. treatment, were therefore transferred to media containing 2 and 4 per cent. glucose, and 2 and 4 per cent. sucrose respectively. Ball (1953) has recently pointed out that there is some hydrolysis of sucrose to glucose and fructose during the autoclaving of White's medium or of media containing Knop's solution. In the present experiment, however, after adjusting the medium to pH 6·4 and carefully autoclaving at 110° C. for 20 minutes, there was no detectable hydrolysis.

Since the results were similar to those previously recorded it will not be necessary to discuss them in detail.

From the present standpoint, the principal result was the occurrence of 'water' forms throughout in both sucrose media, while in the glucose media 'water' forms were found only in the lower concentration, the 4 per cent. solution giving rise to 'land' forms in every case. Representative plants from 4 per cent. sucrose and glucose respectively are illustrated in Pl. IV, Figs. 14 and 15.

Development of sporelings supported at surface of medium

A comparison has already been made (Allsopp, 1953*a*) between the growth of sporelings in liquid media and on a 0·75 per cent. agar medium. It was found that although the growth rate was less on the agar medium, and the

heteroblastic development delayed, yet the radial land type of leaf was produced earlier.

Since there was some doubt as to the explanation of these results, further experiments on the development of supported sporelings have been carried out.

Series MD 13/11. Marsilea sporelings from the same sporocarp were grown on the following media, consisting of the basic constituents plus: 2 per cent. glucose; 2 per cent. glucose+0·75 per cent. agar; 2 per cent. glucose with loosely packed cotton-wool reaching the surface of the liquid, 2 per cent. glucose with the same amount of cotton-wool pressed well below the surface, 4 per cent. glucose, and 4 per cent. glucose with cotton-wool reaching the surface.

The results were clearly defined. Of the sporelings immersed in the liquid medium, those in 2 per cent. glucose, and in 2 per cent. glucose with wool at the base of the culture tube, were all of the 'water' form. The latter sporelings from the medium containing cotton-wool had a higher growth rate and produced longer internodes than those from the sugar-only medium, indicating that some growth factor is supplied by the cotton-wool. In the 4 per cent. glucose solution seven sporelings out of a total of ten had distinct land-form characteristics.

Of the sporelings growing at the surface of the medium, those from 2 per cent. glucose+cotton-wool were well grown and included eight 'land' forms from a total of ten sporelings. The sporelings on agar were all 'land' forms, but considerably stunted as compared with those on cotton-wool. On 4 per cent. glucose+wool all sporelings were 'land' forms and more stunted than those in the corresponding liquid medium.

The results of this experiment indicate quite clearly that exposure of the normal aerial parts above the liquid tends to promote the appearance of land-form characteristics.

Transfer experiments

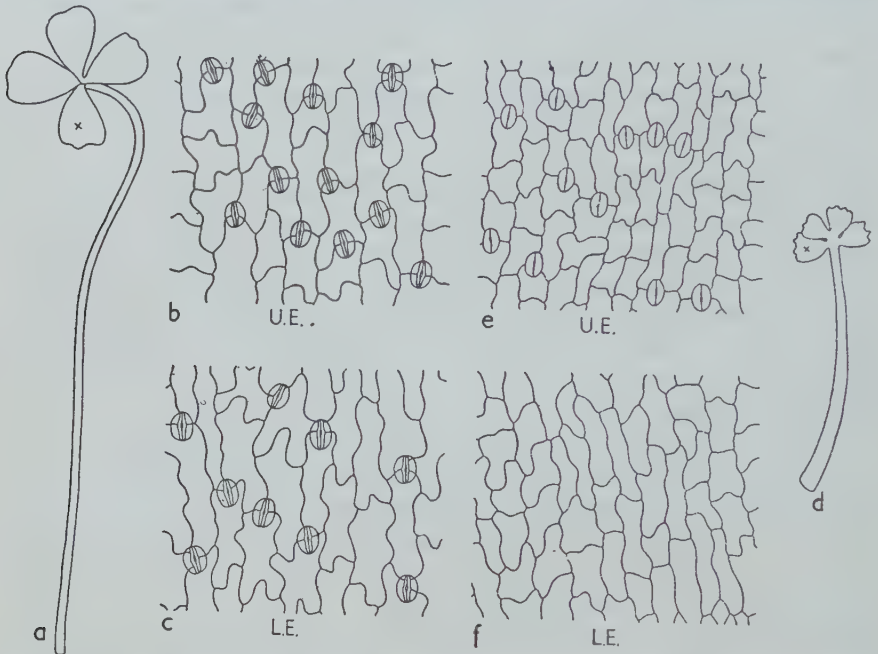
In the experiments described above, the sporelings were exposed to the action of a single culture media during the whole of their period of development. The present section deals with the effects of the transfer to a second medium of sporelings which had already undergone considerable development on the initial medium.

(*a*) *From high to low concentrations of sugar*

Several experiments in which sporelings were transferred to lower sugar concentrations all yielded essentially the same result.

In the first experiment of this series four sporelings with typical land-form characteristics were transferred from a medium containing 5 per cent. glucose to the basic Knop's solution without any sugar. During the further growth of each plant there was an immediate change to the 'water' form, apart from a lack of internode extension, which was attributed to a failure in the carbohydrate

supply. This interpretation is supported by the fact that even typical 'water' forms from 1 and 2 per cent. glucose underwent a shortening and final disappearance of internodes when transferred to Knop's solution (Allsopp, 1953). A representative sporeling showing the abrupt change from 'land' to 'water' form is illustrated in Pl. IV, Fig. 16. It was found that leaves already fairly well developed at the time of transfer gave rise to a somewhat transitional



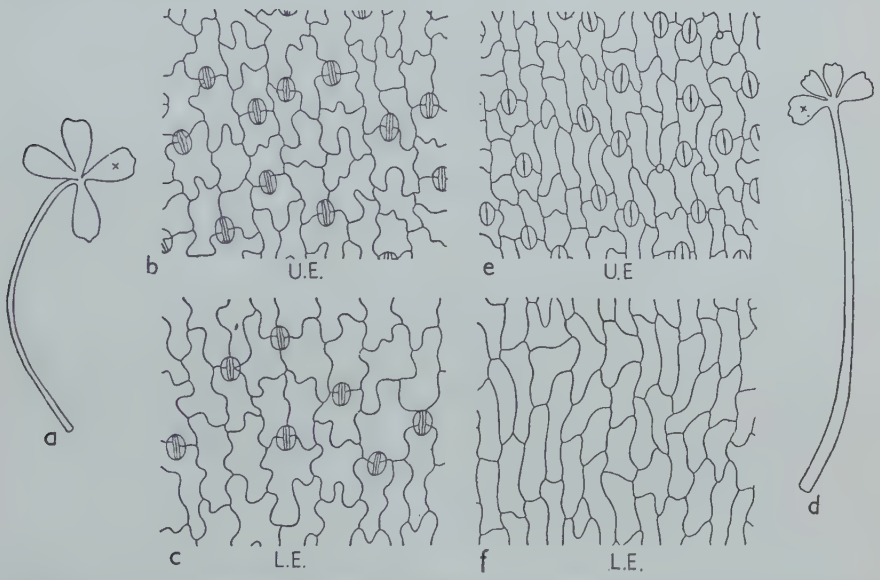
TEXT-FIG. 4. *M. Drummondii*. Effect of transfer of sporeling from medium containing 5% glucose to basic inorganic medium only. (*a*), (*b*), (*c*) typical leaf before transfer with upper and lower epidermis from part marked X. (*d*), (*e*), (*f*) corresponding figures for third leaf formed after transfer. Leaves $\times 2\frac{1}{2}$. Epidermis $\times 157$.

form of leaf, but that young leaf primordia underwent a complete change to the type of leaf characteristic for the new medium.

Anatomical Effects. Anatomical study was restricted to the leaf, since it was this organ which showed the most evident response to the change in culture medium. A typical leaf, before transfer, of the plant shown in Pl. IV, Fig. 16, is illustrated in Text-fig. 4*a*, and parts of the upper and lower epidermis in Text-fig. 4, *b* and *c*. The structure is clearly that characteristic of a plant from high sugar concentrations. Text-fig. 4, *d*, *e*, and *f* represent the third leaf developed after the transfer from 5 per cent. glucose to the purely inorganic medium. This leaf has all the characteristics of leaves normally developed in low sugar concentrations, with restriction of stomata to the upper epidermis, reduction in the number of stomata in the latter, and lack of marked cuticular thickening or depression of the guard cells.

In the above experiment the principal morphological changes following

transfer were too abrupt to suggest that they were a consequence of carbohydrate starvation. This interpretation of the results was tested by another experiment in which two plants were transferred from a medium containing 5 per cent. glucose to a medium with only 2 per cent. glucose. As in the previous experiment, there was an immediate change to the 'water' form, but the presence of 2 per cent. glucose permitted normal internode extension. One of these plants, three weeks after transfer, is shown in Pl. IV, Fig. 17. The other plant persisted as a 'water' form during the remaining four months of growth.



TEXT-FIG. 5. *M. Drummondii.* Effect of transfer of sporeling from medium containing 5% glucose to medium with 2% glucose. (*a*), (*b*), (*c*) typical leaf before transfer with upper and lower epidermis from part marked X. (*d*), (*e*), (*f*) corresponding figures for second leaf formed after transfer. Leaves $\times 2\frac{1}{2}$. Epidermis $\times 157$.

The change in morphology following transfer from high to low sugar concentrations was certainly not a result of carbohydrate starvation, since, as shown by the photograph, growth after transfer was more vigorous than before.

Other experiments in which plants were transferred from 4 per cent. glucose to 2 per cent. yielded virtually identical results. Anatomical examination showed that as in the previous experiment the leaves produced after transfer had the stomatal distribution characteristic of the 'water' form. Text-fig. 5 illustrates the upper and lower epidermis of leaves produced before and after transfer by the plant shown in Pl. IV, Fig. 17.

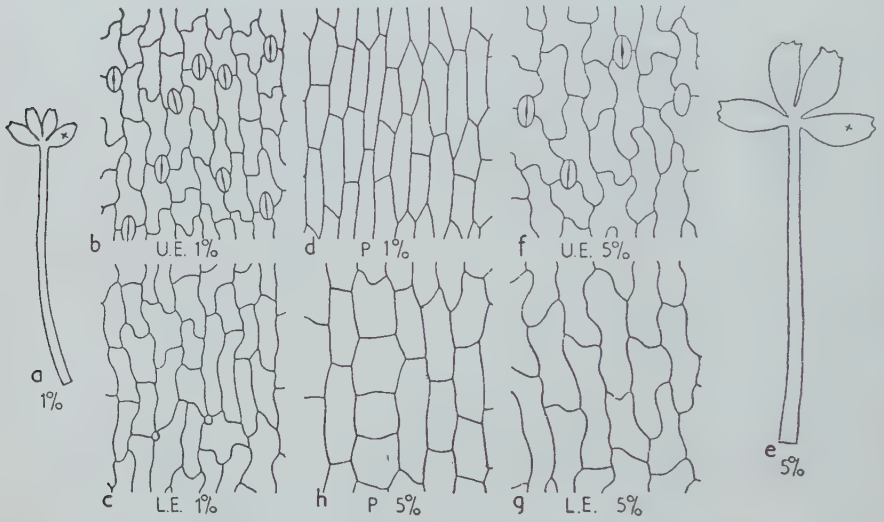
(*b*) *From low to high concentrations of sugar*

It would be a reasonable expectation from the results of the experiments described in section (*a*), that transfer of sporelings from low to high concentrations of sugar would result in an abrupt change in morphology to the type

characteristic of sporelings grown in high sugar concentrations from the outset. It has been found, however, that although there is indeed an immediate response, it is not of the kind suggested.

In the first experiment of the present section two plants were transferred from a medium containing 1 per cent. glucose to one with 5 per cent. glucose. A photograph of one of the plants three weeks after transfer is shown in Pl. IV, Fig. 18. The other plant showed the same response.

It is apparent from the photograph that although there was a considerable increase in the length of the petiole and the size of the lamina of the leaves



Text-fig. 6. *M. Drummondii.* Effect of transfer of sporeling from medium containing 1% glucose to medium with 5%. (*a*), (*b*), (*c*), (*d*) third leaf before transfer, with upper and lower epidermis of lamina, from part marked X, and epidermis of petiole. (*e*), (*f*), (*g*), (*h*) corresponding figures for second leaf formed after transfer. Leaves ×2·5. Epidermis ×157.

formed after transfer, yet the characters are still those of a 'water' form, with non-radial leaves and elongated internodes in the rhizome.

Examination of the epidermal cells of the leaves showed that a marked increase in the size of the cells in the higher sugar solution is sufficient to account for the greater size of the lamina. In the petiole the cells were appreciably wider in the higher sugar concentration, but of approximately the same length, indicating that an increase in the number of transverse cell-divisions was responsible for the greater length of the organ. Representative parts of the epidermis of petiole and lamina of leaves before and after transfer are illustrated in Text-fig. 6. It is apparent from the figure that there was no change in stomatal distribution following transfer.

A similar experiment in which three plants were transferred from a 2 per cent. to a 5 per cent. glucose medium gave essentially the same results. The plants continued to produce leaves of the 'water' form for a considerable

period, but eventually gave rise to radial leaves, although the long internodes of the rhizome were retained. On transfer of sporelings from the normal 2 per cent. glucose medium to a 5 per cent. glucose medium from which all inorganic constituents were omitted, there was again no change to the 'land' form, but in this series of cultures there was no increase in size following the transfer.

In another experiment in which apices of plants grown in 2 and 3 per cent. glucose media were transferred to a medium containing 7·5 per cent. glucose a considerable number of non-radial 'water' leaves were formed before the eventual appearance of typical 'land' leaves.

(*c*) *From air to liquid*

A considerable range of experiments was carried out in which plants originally growing at the surface of the culture medium were later immersed in a purely liquid medium.

Cultures on cotton-wool. As described earlier in this paper, sporelings growing on cotton-wool had usually the features of 'land' forms even when the concentration of glucose in the medium was only 2 per cent. Such cultures when immersed in a liquid medium of identical composition underwent an immediate change to a 'water' form on further development. Exactly the same type of result was obtained with cultures from cotton-wool+4 per cent. glucose when transferred to a 2 per cent. glucose medium.

On the other hand, when cultures from cotton-wool were immersed in a 5 per cent. glucose medium, there was no change in morphological features.

Agar cultures. As in the case of the cultures from cotton-wool, the 'land' forms from agar immediately developed the characteristics of 'water' forms when immersed in a 2 per cent. glucose liquid medium, while in a 5 per cent. glucose medium the 'land' form was retained.

(*d*) *From glucose media to media containing mannitol*

In the present section transfer was made to media in which the osmotic concentration was changed by addition of mannitol.

The first set of transfers was to a medium containing 2 per cent. glucose+3 per cent. mannitol, i.e. a medium of approximately the same osmotic concentration as a 5 per cent. glucose medium. As described earlier in the present paper, sporelings growing on such a medium from the outset were abnormal and stunted, but the well-developed sporelings used in the transfer experiment continued normal growth for a period of weeks. Sporelings with 'land' form characteristics, from 2 per cent. glucose+agar, 4 per cent. glucose, and 4 per cent. glucose+cotton-wool, retained these features after transfer to the mannitol medium, while 'water' forms from 2 per cent. glucose also retained their original features. In contrast with the sporelings transferred from 2 to 5 per cent. glucose media, there was no marked increase in size on transfer to 2 per cent. glucose+3 per cent. mannitol, indicating that the size effect in the 5 per cent. glucose medium was of nutritional rather than

osmotic origin. When the inorganic constituents were omitted from the 2 per cent. glucose+3 per cent. mannitol medium, the results were essentially similar, apart from an earlier arrest of growth.

Discussion

The results of the present investigation, taken in conjunction with those described in previous contributions of the series, enable certain conclusions to be drawn with reference to two aspects of morphogenesis in *Marsilea*: (*a*) heteroblastic development, and (*b*) the origin of land or water forms.

From the earlier work it was concluded that the heteroblastic development is a response to the increase in size of the apex of the developing sporeling; the size of the apex is in turn dependent on the nutritional status of the plant, which thus controls the type of leaf produced. None of the work described in the present paper is in conflict with this hypothesis, and, in particular, the series of experiments in which mannitol was added to the culture medium has shown that the higher rate of heteroblastic development in sugar solutions of increasing concentration is not merely a result of the greater osmotic concentration of the medium. Other evidence in support of the nutritional view of heteroblastic development has been summarized in a recent discussion (Allsopp, 1954*b*).

The problem of the origin of land or water forms, especially the nature of the factors responsible for the characteristic morphological features of water plants, has given rise to much discussion, but there is still no general agreement. The literature is too extensive to review here, but the principal views may be indicated. Askenasy (1870) carried out the first experimental work on the subject, but like other workers of that period he considered that the characteristic features of water plants arise as adaptations which render these plants more suitable for life in the aquatic medium. Goebel (1891) rejected this teleological approach and advanced the hypothesis that the characteristics of submerged plants are a response to a deficiency of assimilates, occasioned by a reduction in light intensity. At present this is perhaps the most widely held view and has been supported by a large number of investigators, e.g. Burns (1904), Glück (1905), Esenbeck (1915), Arber (1920), Riede (1921), Woltereck (1928).

A different explanation, however, was put forward by McCallum (1902) as a result of his extensive investigations on *Proserpinaca palustris*. He concluded that the features of the submerged form are a consequence of the suppression of transpiration leading to increased dilution of the cell sap, which is followed by changes in cellular extension and division. This hypothesis received little support for a long period, but similar views have now been put forward by Combes and his colleagues, whose extensive work on the morphological and physiological effects of changes in environment has been summarized recently (Combes, 1946). In an important contribution Gertrude (1937) made a thorough analysis of the effects of a change of medium on *Veronica anagallis*. She found that, contrary to what is usually maintained,

photosynthesis in plants grown in water is not less than in air but is at least equal. Furthermore, as judged on the basis of protein content, the water forms actually contained more living matter than the plants grown on land. In the water plants there was a lower concentration of soluble carbohydrates, which was certainly not due to a lower rate of photosynthesis, but was a result of the further transformation of these products in the synthesis of proteins, &c. Gertrude concluded that the characteristic morphology of water plants is an effect of the low content of the tissues in soluble carbohydrates, and of the low osmotic concentration produced by both the high water and low sugar content. Strong support for this view was provided by Combes (1936), who by growing *Veronica anagallis* aseptically in 10 per cent. glucose solutions obtained plants with all the characteristics of the normal land form.

The somewhat revolutionary findings of Gertrude were confirmed by Gessner (1940), who found that in *Ranunculus baudotii*, photosynthesis was as active in plants grown in water as in those on land, but that only a small proportion of the assimilates persisted as carbohydrates. He also discovered that the water type of leaf could be obtained even on land by removal of all carbon dioxide from the atmosphere. On the basis of these and other results Gessner advanced a new and somewhat curious hypothesis, which attempted to reconcile the views of the French and German schools. The formation of the water form of leaf was referred to reduced photosynthesis in the young leaves of the bud which are more closely packed in the water form. Even from existing knowledge of the dependence of bud expansion on translocation from other parts of the plant, one can attach little significance to this hypothesis. It has also been disproved experimentally by the subsequent work of Bauer (1952), who demonstrated that the land form of leaf could be obtained from darkened buds placed under water, provided that the other parts of the plant remained on land. He concluded that the leaf form of *Ranunculus aquatilis* is determined by metabolic processes in the bud which are limited by the intensity of photosynthesis in the leaves. Bauer also considered that differences in the behaviour of different experimental plants may account in part for the opposed views of the various schools.

It would seem, however, that certain general principles emerge from a consideration of the existing literature and the experimental results now obtained with *Marsilea*. In the first place, the work of Gertrude (1937) and Gessner (1940) has shown clearly that water forms do not necessarily photosynthesize less actively than land forms. Consequently the features of water forms are not a response to a diminished supply of nutrients. This view is supported by the present work on *Marsilea* in which very vigorous sporelings, for example, those stimulated by addition of 10 mg./l. of indolylacetonitrile to the culture medium (Allsopp, 1954*a*), are frequently of the 'water' form, while stunted sporelings, for example those on agar media, may have 'land'-form characteristics. It is indeed found that in a batch of sporelings cultured under identical conditions, the less actively growing individuals have a greater tendency to give rise to the 'land' form.

The evidence points rather to the water-saturation of submerged plants as the factor responsible for the appearance of the characteristic features of water forms. Thus, in the present work, 'land' forms of *Marsilea* were obtained when sporelings were supported at the surface of a medium which gave rise to 'water' forms in submerged sporelings. In this experiment nutritional deficiency in the 'water' form was excluded by the supply of an organic carbon source. The importance of a water deficit in the production of land forms is emphasized by the experiments on *Marsilea* in which 'land' forms were obtained even in submerged sporelings by raising the osmotic concentration of the culture medium. These experiments also rule out the view that 'water' forms develop in response to oxygen deficiency.

Although it seems clear from the above considerations that under natural conditions it is the water relations of the environment rather than the nutritional status of the plant that determine the occurrence of land or water forms, the way in which this factor operates is less evident. At least two important internal effects of water supply must be taken into account in submerged plants, namely a decrease in osmotic pressure and a decrease in sugar concentration, and since changes in these factors frequently follow a similar course, it is not easy to separate their action. It is possible that the morphological effects of the aquatic environment are a consequence of changes in the protoplasmic colloids in response to changes in the osmotic concentration of the cell sap. It is also possible that we are dealing with a direct response to the higher sugar concentrations as such rather than with the associated osmotic consequences. As a third possibility both factors might operate simultaneously.

The available evidence is not decisive, although certain results suggest that sugar concentration may be more important than osmotic pressure. Thus sporelings grown in a medium containing 5 per cent. glucose were always 'land' forms, while sporelings from a medium containing 2 per cent. glucose with addition of 3 per cent. mannitol were mainly 'water' forms. The hypothesis that it is the sugar concentration that determines the type of morphological expression enables us to explain other results. Thus, it is known that under conditions of intensive lighting the land type of leaf may arise even in submerged plants. A formation of sugars in photosynthesis at a rate greater than that of their utilization would account for this observation. In the transfer experiments with *Marsilea* the immediate change to the 'water' form on transfer from a high to a low sugar medium would be accounted for on either view by rapid entry of water into the growing regions. The delayed appearance of the 'land' form following a transfer from low to high sugar media could be referred to a gradual building up to the required sugar concentration, but is perhaps more readily explained by the assumption that before transfer the low sugar concentration was limiting growth and mineral nutrients accumulated. Following transfer, the reserve of mineral nutrients would favour a rapid synthesis of protoplasmic materials, and thus delay the increase in sugar concentration necessary for the appearance of the 'land' form.

Whichever view one adopts to account for the observed morphological effects of the osmotic pressure (or more generally, diffusion pressure deficit) of the environment, the difficulty of accounting for the numerous and varied responses to a relatively simple change still remains. In the present work a gradual increase in the concentration of the culture medium has not led to the appearance of plants intermediate between land and 'water' forms. Instead there has been an abrupt change, once a limiting concentration has been passed. It would seem that when a certain internal osmotic pressure (or sugar concentration) is attained, some 'trigger-mechanism' is set in motion releasing a whole series of specific reactions, such as appearance of palisade cells in the leaf, formation of stomata in the lower epidermis, &c. As shown in an earlier paper (Allsopp, 1954), many of these morphological differences may be referred to changes in the pattern of cell division in the shoot apex. But although the detailed mechanism of the process is still obscure, there is little doubt that it is the water relations of the environment, rather than nutritional conditions, which determine whether land or water forms develop.

Acknowledgements

I wish to thank Professor C. W. Wardlaw for his encouragement and advice, and Mr. Ernest Ashby for the photographs in Pls. III and IV.

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DESCRIPTION OF PLATES

Illustrating A. Allsopp's article on 'Experimental and Analytical Studies of Pteridophytes. XXVII. Investigations on *Marsilea*. 5'.

PLATE III. *Marsilea Drummondii*

Figs. 1–6. Representative sporelings from same sporocarp grown for 4 months on media containing (1) 2% glucose, (2) 4% glucose, (3) and (4) 2% glucose+2% mannitol, (5) 2% glucose+4% mannitol, (6) 4% sucrose+3% mannitol. (All nat. size.)

Figs. 7–11. Sections of plant shown in Fig. 3. (7) T.S. of lamina (×200), (8) T.S. petiole (×75), (9) T.S. root (×100), (10) T.S. rhizome (×75), (11) Median L.S. of rhizome apex (×150).

PLATE IV. *Marsilea Drummondii*

Figs. 12, 13. Sporelings after 6 months of growth on media containing 2% glucose, but (12) 10× and (13) 5× the normal concentration of Knop's solution.

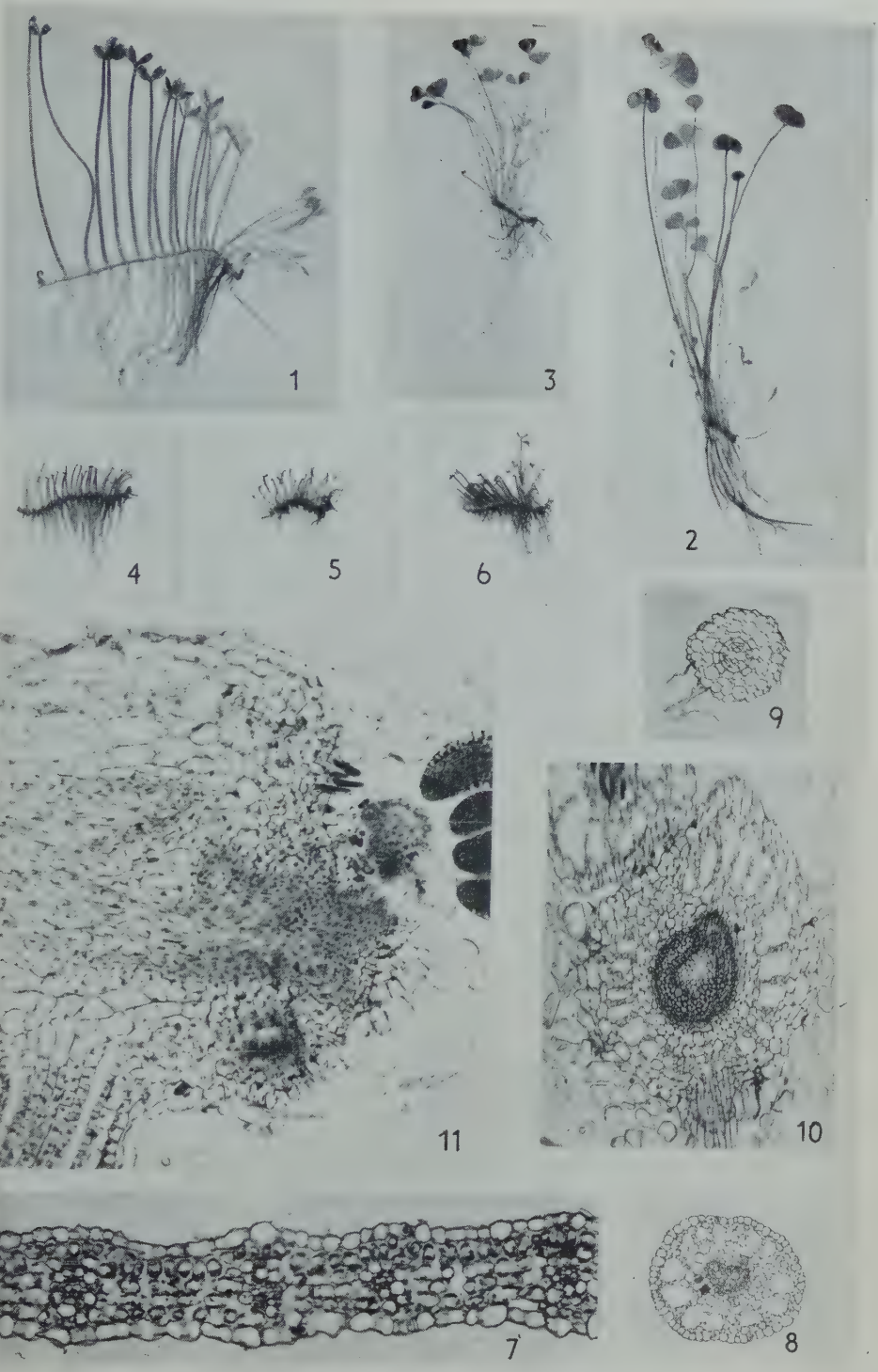
Figs. 14, 15. Sporelings grown for 4 months on media containing (14) 4% sucrose and (15) 4% glucose.

Fig. 16. Sporeling 3 weeks after transfer from 5% glucose medium to medium without sugar. Parts on left of arrow developed after transfer.

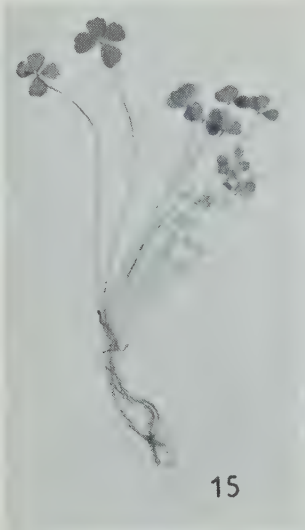
Fig. 17. Sporeling 3 weeks after transfer from 5% glucose medium to medium with 2% glucose. Parts on left of arrow developed after transfer.

Fig. 18. Sporeling 3 weeks after transfer from 1% glucose medium to medium with 5% glucose. The abrupt increase in size of the leaves indicates the parts formed after transfer.

Figs. 12–18 natural size.



A. ALLSOPP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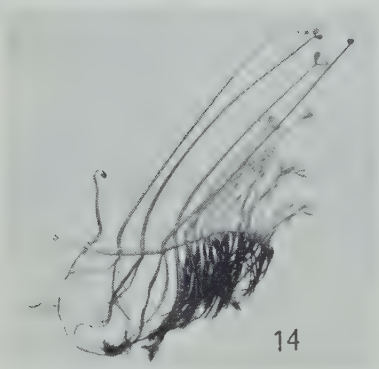
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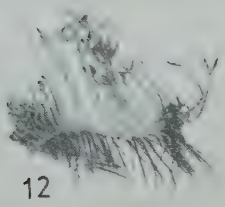
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A. ALLSOPP

Studies of Growth and Development in the Genus *Fragaria*

IV. Winter Growth

BY

S. E. ARNEY

(*University College, Cardiff*)

With one Figure in the Text

Abstract

The initiation and growth of leaf primordia has been followed during four winters, and similar rates were obtained in each season. When allowance is made for temperature differences, these rates are at a much lower level than during the active growing season. In this respect winter dormancy involves a physiological change resulting in a lower level of activity rather than a complete cessation of activity. Leaf emergence and expansion, on the other hand, is completely stopped during the winter; this appears to be the result of photoperiodic induction by the shortening days of August and September and is not primarily dependent on the fall in temperature.

Introduction

SEASONAL and climatic changes in environment throughout the growing season do not alter the proportion between the rate of leaf initiation and the subsequent growth rate of the leaf initials in 'Royal Sovereign' strawberry plants, and this balance maintains a constant number of 6[1] leaf initials enclosed within the apex (Arney, 1953). Exceptions were noted in early spring, when the plants had 7–8 leaf initials enclosed within the apex, and in May, when there were only 5 enclosed initials. It seemed likely that further investigation of these exceptional conditions might throw fresh light on the nature of the correlation between leaf initiation and leaf expansion which maintains this constant number of enclosed leaf initials throughout most of the growing season. The two exceptional cases will be treated separately, since they are shown to be the results of different growth conditions during winter and spring.

Leaf Initiation during the Winter

Fresh leaves do not usually emerge on 'Royal Sovereign' plants during the months of December and January, and the few that do emerge at this time do not expand until the spring. The proportion of plants in which a leaf emerges during these winter months seems to depend upon the severity of

[1] N.B. The normal number of leaf initials within the bud was previously given as 5, *excluding* the emergent leaf (Arney, 1953). For the purposes of this study it is simpler to *include* the emergent leaf as one of the enclosed initials, making all the corresponding numbers greater by one.

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the season, and therefore on the locality. No leaves emerged on any plants out of doors at Cardiff during December and January 1952–3, as the winter was exceptionally severe; but in milder winters a small proportion of plants may produce one leaf during these two months. According to Ball and Mann (1926) no root growth occurs in strawberry plants during the latter part of December or in January. Thus the plants give every appearance of winter dormancy.

Table I gives the mean number of enclosed leaf initials in samples of 10–15 plants dissected on each occasion at intervals over several growing

TABLE I

Winter Leaf Initiation and Number of Enclosed Leaf Initials

A. Number of enclosed leaf initials, including the emergent leaf.
B. Number of leaves initiated since the previous dissection.
C. Plastochron interval (in days) based on leaf initiation.
(No leaves emerged and expanded during this period on any of these plants except under long-day treatment, with supplementary illumination.)

		A.	B.	C.
Winter 1950–1:	Nov. 11, 1950	5·8	—	68
	Jan. 25, 1951	6·9	1·1	
Winter 1951–2:	Nov. 9, 1951	6·1	—	90
	Jan. 29, 1952	7·0	0·9	
Winter 1952–3:	Dec. 4, 1952	6·5	—	88
	Jan. 17, 1953	7·0	0·5	
	Feb. 17, 1953	7·4	0·4	78
Winter 1953–4:	Nov. 9, 1953	6·5	—	50
	Dec. 4, 1953	7·0	0·5	
	Jan. 9, 1954	7·1	0·1	360
	Feb. 26, 1954	7·1	0·0	

seasons. There is conclusive evidence that most plants initiate one leaf between the middle of November and the middle of January, but the suggestion in the 1952–3 results that the leaf may be initiated at any time during this period is not confirmed by the 1953–4 results. The regularity with which the mean value for number of enclosed leaf initials on each occasion changes throughout the season, and the close correspondence between the different years, makes it unlikely that the difference between the two seasons 1952–3 and 1953–4 is due to sampling errors. The mean numbers of enclosed leaf initials during the winters of 1950–1–2–3–4 agree very closely indeed, and column C shows that there is a close agreement between the rates of leaf initiation for these three winters.

Of the 10 plants dissected in December 1952, only 3 had initiated a leaf primordium after the second inflorescence primordium; the other 7 plants had this second inflorescence as the last formed primordium. In January 1953 only 3 plants had no leaf primordia formed after the second inflorescence, while 12 had already formed another leaf primordium after this inflorescence; in February all 9 of the plants to which this analysis could be applied had

leaf primordia formed after the second inflorescence. The χ^2 test shows the former difference to be well above the 5 per cent. level, but the difference between January and February is not significant; when all three dates are compared the differences are significant beyond the 0·1 per cent. level. This analysis confirms that leaf primordia were initiated during December and January 1952–3. A similar analysis for the 1953–4 winter shows that all plants had already produced 2 or more leaf initials after the second inflorescence by the beginning of December, and no more than this by the end of February, thus confirming the inference from Table I that leaf initiation ceased at the beginning of December 1953, having proceeded unusually rapidly during November 1953. This unusual prolongation of comparatively rapid leaf and inflorescence initiation throughout November, without corresponding leaf emergence, resulted in the abnormally high number of enclosed leaf initials at the beginning of December (Table I), and also in the initiation of a greater number of inflorescences per plant (see later section). No leaves emerged between December 1953 and February 1954 on any of the plants dissected during this period, so the failure to increase the number of enclosed leaf initials after the beginning of December is not the result of emergence of the oldest initial during this time.

There were marked climatic differences between the winters of 1952–3 and 1953–4, and these probably caused the different behaviour of the plants in the two seasons; but the relation is not straightforward, and will be discussed in subsequent sections.

Inflorescence Initiation

The initiation of inflorescences in 'Royal Sovereign' plants commences early in September and continues throughout the autumn, but there is no further initiation of inflorescences after the resumption of active growth in the spring, although the plants are then growing in a shorter photoperiod and at lower temperatures than in September. Plants which are kept in active growth throughout the winter in warm conditions and long days will continue to initiate inflorescences throughout the winter, spring, and early summer (unpublished experiments, and also Darrow and Waldo, 1934). The transition from inflorescence initiation to vegetative growth therefore appears to be associated with the winter rest period, and it is of interest to determine more exactly the time at which inflorescence initiation ceases. Of the 112 crowns which were dissected during the months of December, January, and February in various seasons, 107 had not initiated an inflorescence since the beginning of December. In 4 of the remaining 5 apices the inflorescence was sufficiently advanced in development that it could reasonably be supposed to have been initiated before the beginning of December. Even in the one remaining case it is not certain that the inflorescence was initiated after the beginning of December; since the winter growth rate is so low, it seems quite possible that 1 crown in 112 might become completely inactive during the winter, and if an inflorescence had been initiated in late November immediately

before inactivity commenced, this crown would appear to have initiated an inflorescence during the winter if it was not dissected until January or February. Thus even the one crown which appears most conclusively to have initiated an inflorescence since December may not, in fact, have done so. There is thus no conclusive evidence that any crown initiates an inflorescence after the beginning of December, and it is quite certain that at least 95 per cent. of plants do not initiate inflorescences after the beginning of December. As this is the time at which the leaf initiation rate of the apex drops to a much lower value, it seems that the transition back to the vegetative condition from floral initiation occurs at the termination of active growth for the season.

The number of inflorescences initiated each year by each plant might be expected to depend upon the duration of the active growth period after floral *induction* is completed. During the autumn of 1952 no plants produced more than two inflorescences per crown, but in 1953 a third inflorescence was present in more than half the total number of crowns dissected. The abnormally mild autumn of 1953 and the prolongation of active leaf and inflorescence initiation until the beginning of December afford an obvious explanation of this difference. Guttridge (1951) found that 'Royal Sovereign' plants varied between 2 and 3 inflorescences per crown at Long Ashton in 1951, which agrees well with the condition of runners grown at Cardiff in that year. But there is some evidence that runner plants obtained from different nurseries do differ consistently in the number of inflorescences per crown, and the causes of this variation are under investigation.

Elongation Growth of Leaf Initials

The change in length of leaf initials during the winter can be estimated by comparing the mean lengths of corresponding initials on apices dissected at intervals throughout the winter. Since no leaves emerge during this period, the serial order of the successive leaf initials from the oldest enclosed initial can be used to establish correspondence. But in determining this serial order it is important to allot a place to each inflorescence as well as to the leaf initials, for there is an exceptionally big difference in length of the leaf initials immediately before and after the inflorescence (Arney, 1953); by including the inflorescences as initials for the purpose of determining the serial order, the lengths of the leaf initials occupying the same serial order do correspond fairly closely in every apex dissected on any one occasion, in spite of the irregularity in size gradation adjacent to each inflorescence. Since there are 2 or 3 inflorescence initials in each bud as well as 7 or 8 leaf initials, Table II contains 10 columns to cover the series within the buds.

From the figures in Table II it is clear that the oldest enclosed leaf initial does not increase in length during any of the three winters covered. Analysis of variance for the pooled results of all the younger leaf initials in 1951–2 shows that the increase in length is significant beyond the 1 per cent. level; but only the differences for the three youngest initials after P.1 are individually

significant. During the more severe winter of 1952–3 the older initials do not elongate at all, but the three youngest initials show a highly significant increase in length between December and February when considered together; the youngest leaf primordium in December becomes the next to youngest in January or February, and this is the comparison to be made in estimating the growth between these dates. November 1953 was exceptionally mild (Table V), and all except the two oldest leaf initials elongated considerably during the month. Between December 1953 and February 1954 there is no statistically significant increase in length of even the youngest primordia, and Table II

TABLE II

Elongation of Leaf Initials during the Winter

In compiling this table the inflorescence initials as well as the leaf initials have been allotted places in the serial order of initials. The youngest leaf primordium in each apex has been placed in the 'youngest' column, irrespective of the total number of initials between it and the emergent leaf. Where the apices had no initials except the youngest beyond a certain serial number, the spaces have been left blank; a rule indicates that no measurements were made although leaf initials were present in that position.

Lengths (in mm.) of Progressively Younger Leaf Initials

(Means of between 10 and 20 apices)

	Emergent leaf.									Youngest primordium.
Nov. 9, 1951	35	17	10	5·4	3·8	1·3	0·48			0·12
Jan. 30, 1952	27	20	14	8·9	4·7	3·4	1·9	0·58		—
Dec. 4, 1952	28	17	9	6·5	4·6	1·8	0·67			0·04
Jan. 17, 1953	27	16	9	5·5	4·1	2·0	0·60	0·10		0·04
Feb. 17, 1953	28	17	10	5·7	4·4	2·9	0·97	0·57		0·06
Nov. 8, 1953	30	15	9	6·1	3·3	1·4	0·51	0·35		0·07
Dec. 8, 1953	27	17	14	9·5	6·4	4·0	1·8	0·64	0·32	0·10
Jan. 9, 1954	26	19	14	10·0	7·3	4·2	1·5	0·85	0·42	0·12
Feb. 17, 1954	28	18	14	9·7	7·1	3·8	1·7	0·82	0·41	0·13

shows that if slight growth did occur during this time it was confined to the month of December, which was also exceptionally mild. In the three winters covered by the observations there is appreciable growth of the younger leaf initials over some part of the winter, in two seasons over almost the whole winter. It is not possible to be certain, however, that there is not a short period of complete inactivity even in the milder winters, but the results for 1952–3 make this somewhat unlikely.

The Relation between Leaf Initiation and Growth of Leaf Initials

One of the chief objects of this further study of winter behaviour of the strawberry apex was to throw fresh light on the relation between leaf initiation and the growth and emergence of the leaf initials. Difference in behaviour in the different winters provides further evidence of a close relationship between the two processes. The period from December 1953 to February 1954 is the only occasion on which leaf initiation has been observed to stop completely,

and it is the only period in which further growth of the leaf initials is known to have stopped too. In contrast, both leaf initiation and the growth of leaf initials continued uninterruptedly, although at a very slow rate, from December 1952 until February 1953. In no case has leaf initiation been observed to occur without simultaneous growth of all the younger leaf initials. The relative sizes of the successive leaf initials in the winter apices in both seasons show that the normal summer relation between leaf initiation rate and the

TABLE III

Primordium Length Ratios During the Winter

The primordium length ratio is the quotient obtained by dividing the length of any particular leaf initial by the length of the next younger leaf initial on the apex. When an inflorescence is intercalated between two leaf initials the value of this P.L.R. has not been included in the mean. Snow's terminology is used, P.1 being the last initiated leaf primordium, and P.2 the next youngest leaf initial on the apex, &c., but P.7 is always the emergent leaf and where there are only 5 enclosed leaf initials in addition to the emergent leaf, P.3 is omitted, and no value for P.2/P.3 is contributed to the mean of the P.2/P.3 P.L.R.

Normal plants.	P.7./P.6.	P.6./P.5.	P.5./P.4.	P.4./P.3.	P.3./P.2.	P.2./P.1.
Aug.–Sept. 1952	2·9	2·0	2·6	2·7	2·7	7·7
Dec. 4, 1952	1·6	1·8	2·0	3·2	3·0	6·0
Jan. 4, 1953	1·7	1·7	—	2·5	3·9	10·0
Feb. 17, 1953	1·7	1·6	2·1	2·9	4·0	13·3
Sept. 10, 1953	2·7	2·0	2·4	2·7	4·4	7·0
Nov. 9, 1953	2·0	1·8	2·1	4·0	4·1	8·4
Dec. 8, 1953	1·6	1·5	2·0	2·2	4·0	6·3
Jan. 9, 1954	1·4	1·5	2·1	2·3	3·7	10·8
Feb. 1954	1·6	1·5	2·3	2·7	4·4	5·6
Long days outside.						
Sept. 10, 1953	3·2	2·0	2·8	3·2	—	6·8
Oct. 9, 1953	2·8	2·2	3·1	4·4	—	4·1
Nov. 9, 1953	2·6	1·8	2·7	3·9	3·8	—
Dec. 9, 1953	2·2	2·0	2·7	3·6	—	4·5

growth rate of the leaf initials is maintained in all except the oldest leaf initials. This follows from the fact that the primordium length ratios (Table III) for the winter months are not very different from those for August and September, except for the oldest three leaf initials. (For explanation see Arney, 1953, p. 486). The oldest two or three leaf initials have much lower primordium length ratios during the winter, which indicates that their growth rates have decreased relative to the leaf initiation rate at the apex. This means that the increased number of leaf initials in the winter apices is not the result of a change in the balance between the growth rate of all the leaf initials and the rate of leaf initiation. Rather, it seems to result from the slowing up of the growth rates of only the older enclosed leaf initials, which prevents the oldest initial from emerging. These facts are fully consistent with the suggestion made in a previous paper (Arney, 1953) that the balance between initiation rate and growth rate of the younger leaf initials depends only on the fact that

both rates are based upon cell division rates in similar meristematic tissues. There does not appear to be any need to postulate a particular co-ordination mechanism, or a hormonal method of control, such as might be expected at first sight from the constancy of the number of enclosed leaf initials throughout a large part of the growing season. But one point needs further study in this connexion: the initiation and growth of leaves ceased at the beginning of December 1953 although the whole of December was exceptionally mild—the first frost of the autumn and winter occurred on December 31; moreover, the cessation of leaf growth and leaf initiation in January and February 1954 contrasts with the continuation of both processes during the corresponding period in 1953 under almost identical temperature conditions (Table V). This complete cessation of growth in December 1953 might be associated with the very low daylight intensity (inferred from the mean daily hours of bright sunlight, Table V). But it may also be that there is a maximum number of enclosed leaf initials of approximately 7, beyond which no further leaf initiation can proceed, and if this is so it will involve a modification of the proportional growth concept suggested above to include an effect of the oldest enclosed initials on the apex and youngest primordia. Some evidence, however, has already been presented against this view in the case of normal summer plants, (Arney, 1953).

Photoperiodic Control of Leaf Emergence

Consideration of the primordium length ratios in Table III led to the conclusion that the elongation rate of the oldest enclosed leaf initials drops to a very low value during late autumn and winter, and this is responsible for the failure to emerge and expand. Experiments were performed to determine the factor responsible for inhibiting emergence, bearing in mind that Garner and Allard (1923) found long-day treatment effective in delaying dormancy and prolonging the period during which fresh leaves were produced in certain woody plants. Plants growing out in the open in wooden tomato baskets were illuminated from August 14, 1953 onwards each evening by two 1,000-watt flood-lights (tungsten filament lamps) giving a minimum light intensity of 150 f.c. at leaf level. The lights were left on sufficiently long after sunset each day to keep the dark period at 6 hours throughout the autumn and early winter. Comparable plants grown in exactly the same way in wooden baskets were shifted into a greenhouse every evening and replaced in the open each morning during September and October, and were left permanently in the greenhouse day and night, throughout the rest of the winter, at an average minimum night temperature of 48° F. These plants received no supplementary illumination and are referred to as short-day warm (S.D.W.) plants. In Table IV the two sets of plants—the long-day cool (L.D.C.) plants and the S.D.W. plants are compared with plants from the normal beds. The mean date of the last leaf to be produced before 'dormancy' indicates that leaf emergence ceased at the same time in both the normal and the S.D.W. plants, but continues

for a week longer in the L.D.C. plants. This difference is significant almost at the 1 per cent. level using the χ^2 test. More leaves emerged on the L.D.C. plants during September, October, and November than on the other two sets of plants, and the number of enclosed initials on the L.D.C. plants at the beginning of December was lower than on the normal plants, being about the same as in summer and early autumn. The χ^2 test shows that these differences

TABLE IV

The Effect of Photoperiod on Autumn Leaf Production

A. Plastochron interval for leaf emergence.
B. Mean date of emergence of the last leaf in 1953.
C. Number of leaves emerged since September 1.
D. Number of enclosed leaf initials.

	A.		B.	C.	D.
	October.	November and December			
Normal outside plants	12	31	Nov. 27	6·9	7·3
Short-day warm plants	15	29	Nov. 26	5·3	5·8
Long-day cool plants					
(*a*) Long days from 14 Aug.	16	20	Dec. 5	7·3	6·3
(*b*) Long days from 8 Oct.	14	23	Nov. 24	—	—

between the two treatments almost reach the 1 per cent. level of significance. This indicates that leaves continued to emerge and expand later in the autumn under long-day treatment, and that there was no accumulation of unemerged leaf initials in the apex. The primordium length ratios for the L.D.C. plants

TABLE V

Winter Climate in 1952 and 1953

		Oct.	Nov.	Dec.	Jan.	Feb.
Monthly mean maximum air temperature	1952–3	55·5	46·6	44·9	42·7	45·0
	1953–4	57·6	53·4	49·2	42·4	44·0
Monthly mean minimum air temperature	1952–3	42·0	38·0	33·5	34·1	35·0
	1953–4	44·0	43·6	41·2	34·3	33·7
Monthly rainfall (in.)	1952–3	1·84	0·12	0·11	1·1	1·96
	1953–4	3·56	3·83	1·73	2·66	4·3
Mean daily hours of bright sunlight	1952–3	3·5	2·8	2·1	1·5	2·6
	1953–4	3·8	1·9	1·2	2·0	3·1

confirm that the older leaf initials continue to elongate and grow in proportion to the younger initials under long-day conditions. But there was a slight decrease in the size difference between the two oldest initials as the autumn advanced, indicating that perhaps the growth of the oldest initial was slightly inhibited, even under long-day conditions.

The primordium length ratios for the intermediate L.D.C. leaf initials (P.3, P.4, and P.5) were consistently higher than the normal summer and

winter values, which may indicate increased growth of these initials too as a result of the long-day treatment. The leaves which emerged in October and November on these L.D.C. plants contained approximately twice as many cells at maturity as the leaves which emerged on normal plants at this time, and they were borne vertically on long petioles as are the normal summer leaves, and unlike the shorter, horizontal petioles of the normal autumn leaves. Since the rate of leaf production, size of the emergent leaf initial, and size of the cells in the emergent leaf initial, are approximately equal in the normal and the L.D.C. plants at this time, the difference in number of cells per leaf cannot be due to differences in cell division rate in the unemerged leaf initials, but must be the result of a prolongation of the cell-division phase during the period of emergence and expansion in the L.D.C. plants. A similar state of affairs occurs in the normal summer leaves (Arney, 1954). The effect of photoperiod on leaf growth is the subject of a further study in this series.

The S.D.W. plants became 'dormant' at least as early as the plants outside under natural photoperiod and temperature and, as in these normal plants, the last leaf to emerge on the S.D.W. plants does not elongate or expand until the following spring. Thus the effect of the additional light on the L.D.C. plants cannot be attributed to any slight increase in night temperature resulting from irradiation by the powerful tungsten filament lamps, for the observed temperature during irradiation did not reach the level of the night temperature in the greenhouse.

When artificial light is not applied to plants in the open until after October 9, it appears to have no effect (see Table IV). This is probably bound up with the induction and incidence of dormancy, which is discussed in the following section, but until the exact nature of dormancy in the strawberry plant is more fully understood it is not possible to interpret fully the significance of these photoperiodic experiments, which were exploratory in nature. Unfortunately the L.D.C. series had to be terminated before the end of December, so that it was not possible to determine whether leaf emergence would have continued during January.

The illumination of plants in the open has been repeated during the autumn and winter of 1954–5, using high pressure mercury-vapour lamps. The experiments, though not yet complete, confirm the previous conclusions about the effect of long-day treatment without artificial heat. Leaves have continued to emerge on the illuminated plants, even during January, although the winter has not been abnormally mild, and has already entailed both frosts and snow. These results suggest that the cessation of leaf emergence and expansion in the late autumn is almost entirely the result of the shortening photoperiod, and not of the falling temperature.

Discussion

The continued activity of strawberry plants during the winter, manifest in the initiation and growth of fresh leaf primordia, is not inconsistent with the prevalent assumption that the strawberry plant enters a period of winter

dormancy, since dormancy rarely, if ever, reaches the stage of complete cessation of all activity. There is ample evidence (Darrow, 1937; Roodenburg, 1939) that strawberry plants which have grown under natural conditions during the early autumn cannot be brought back into vigorous growth merely by applying the normal optimum growth conditions. Long days and temperatures above the normal optimum are required to break this dormancy. Experiments during the past two seasons at Cardiff have not yielded consistent

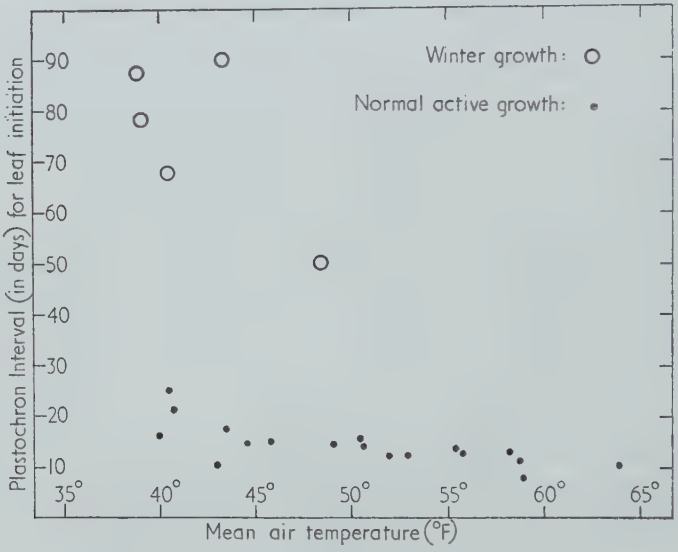

Fig 1. Temperature and heat initiation rate.

confirmation of the existence of this type of dormancy in 'Royal Sovereign' strawberry plants, and further experiments are planned. But the slow winter rates of leaf initiation are below the level which would be expected from fully active plants at comparable temperatures. In Fig. 1 the rates of leaf initiation over different periods during the winters of 1951–4 are plotted against the mean air temperature for the period (this is assumed to lie half-way between the mean maximum air temperature for the period and the mean minimum air temperature for the period). The rates of leaf initiation during the active growing seasons of 1940, 1948, 1949, and 1951 are also plotted against the corresponding mean air temperatures in Fig. 1. It is obvious that the winter rates of leaf initiation do not fall on the same line as the summer rates, and that there is a much lower rate of leaf initiation for any given mean air temperature in the winter dormant condition. This lower level of activity is presumably one aspect of the dormancy phenomena demonstrated by Darrow and by Roodenburg. The alternative assumption that the temperature coefficient of cell division is very high at temperatures below 45° F. does not appear very likely since there is no indication of such an increase in the trend

for the active growing season. Although shoot growth occurs at low temperatures in most vernalisation experiments, it is not often that the growth rate of the apical meristem is recorded actually during the cold treatment. Data obtained with barley (Borthwick, Parker, and Heinze, 1941) indicates that the temperature coefficient of cell division and leaf initiation *decreases* at temperatures below 45° F.

Conclusions

1. The initiation of leaf primordia continues uninterruptedly throughout the winter in many seasons, and the younger leaf initials also continue to grow during the winter. The rate of leaf initiation is much lower than would be expected from the relation between initiation rate and mean air temperature for the active growing season. This lower level of meristematic activity during the winter, after allowance for temperature differences, may be one basis for winter dormancy.

2. The emergence and expansion of leaves, which normally stops in November, can be made to continue into December under the influence of long-day treatment without artificial heat in mild seasons at least. But the long-day treatment must be commenced early in the autumn. This long-day treatment also prolongs the cell division phase in the emerging leaf initials and doubles the number of cells in these leaves at maturity, with a corresponding increase in leaf size.

3. The emergent or almost emergent leaf initials do not grow at all during the winter, and the growth rates of the next oldest leaf initials decline considerably as they approach the size of the oldest initial. But the growth rates of the younger initials retain the normal summer and autumn relation to the growth rate of the apex; when apical growth stops, the growth of the youngest initials also ceases.

4. During the winter each plant produces at least one fresh leaf primordium so that, by February, there are 7 or more enclosed leaf initials on each crown. The initiation of this leaf may occur at any time during the winter, varying with the individual plant.

5. There are good grounds for believing that inflorescence initiation always ceases at the beginning of the winter period of lowered activity of the apex. This is shown to be the case in at least 95 per cent. of the plants dissected.

6. The observed facts of winter growth are not inconsistent with the hypothesis that the relationship between the growth rates of the younger leaf initials and of the apex itself is entirely based on similar rates of cell division in similar meristematic tissue in the two cases. The growth rates of the oldest leaf initials, however, do not bear the same relationship to the growth rate of the apex in the winter as in the summer.

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Studies of Growth and Development in the Genus *Fragaria*

V. Spring Growth

BY

S. E. ARNEY

(*University College, Cardiff*)

ABSTRACT

From observations of leaf emergence dates and periodical dissections of stem apices it appears that leaf initiation does not keep pace with leaf emergence during the spring. This leads to a subnormal number of enclosed leaf initials at each stem apex at some time in April or May. Forced plants behave similarly, and it is concluded from these results and from direct experiments that part of the lag of leaf initiation on leaf emergence is connected in some way with the flowering of the first inflorescence to emerge from the crown. Conditions in the bud at the end of the winter also lead to an excess of leaf emergence over leaf initiation in the early spring.

IN a previous study of the apical growth of 'Royal Sovereign' strawberry plants (Arney, 1953) a definite cycle of changes in the number of enclosed leaf initials was observed to occur during the spring. This cycle has been followed by dissections at frequent intervals throughout two further growing seasons, and the results, together with those of earlier seasons, are given in Table I. The values in this table are the means of upwards of 10 stem apices, and the fractional values indicate the relative numbers of apices with fewer or more enclosed initials than the mean number of enclosed initials. As soon as a leaf initial becomes recognizable, when it is a lateral crescent-shaped ridge projecting rather less than 10 μ from the surface of the apex, it is counted as a whole primordium; each individual apex is always deemed to have a whole number of leaf initials. As a result of the inhibition of leaf emergence during the winter, combined with the continuation of leaf initiation (Arney, 1955), the stem apices contain at least one extra enclosed leaf initial at the commencement of spring growth in February (see Table I). At this time most stem apices have 7 enclosed initials, and a few have either 6 or 8 enclosed leaf initials. After 2 months' active growth and leaf expansion during March and April most of these apices have one less than the normal number of enclosed initials, but since all apices do not drop to the 'minimal condition' of only 5 enclosed initials at the same time, the batch of shoots dissected on any one occasion at this time of the year will contain almost equal numbers of apices with 5 or with 6 enclosed initials.

The fact that not all apices dissected on any one occasion in May have as few as 5 enclosed initials might be explained in other ways. It might be that not all plants undergo the reduction to 5 enclosed initials, and that some apices

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contain at least 6 enclosed initials throughout the whole of the spring. Such a situation could be brought about either through a lower rate of leaf *emergence* in the plants with 6 enclosed initials, thus enabling the rate of leaf initiation to keep pace with leaf emergence, or alternatively through a lower rate of leaf *initiation* in the plants with only 5 enclosed initials. The first alternative can be ruled out from an analysis of the results of dissections performed in May of each year. It was found that the apices which had 6 enclosed initials on these occasions had an average of over 6 leaves already emerged before dissection, while the apices containing only 5 enclosed initials on these occasions had an average of less than 5 leaves already emerged. The second alternative can be ruled out from the results of dissections performed in April of each year, when the plants whose apices contained only 5 enclosed initials had already produced more than 4 emerged leaves each, while plants whose apices contained 6 enclosed initials had produced less than 3 emerged leaves up to the time of dissection.

TABLE I

Change in Number of Enclosed Leaf Initials during the Spring

The values are the means of 10 or more apices. The emergent leaf is included in the number of enclosed initials.

1951.		1952.		1953.		1954.	
Jan. 25	6·9	Jan. 29	7·0	Jan. 17	7·0	Jan. 9	7·1
				Feb. 17	7·4	Feb. 13	7·0
						Feb. 26	7·1
				Mar. 10	7·0		
				Mar. 23	6·6	Mar. 26	5·8
				Apr. 8	6·1		
				Apr. 30	5·9	Apr. 26	5·3
May 3	5·0	May 5	5·0	May 13	5·6	May 7	5·3
		May 26	5·2	May 20	5·6	May 19	5·9
				May 26	6·2	June 3	6·2

The difference between the results of dissections in April and May can be explained immediately in terms of the assumption that all plants pass through the stage in which their apices come to have only 5 enclosed primordia at some time during April or May. When all the apices dissected during April and May 1953 and 1954 are classified according to the number of leaves which have already emerged during the spring up to the time of dissection, and also according to the number of enclosed initials at the time of dissection, the following table is obtained:

Number of enclosed leaf initials.	Number of leaves which have emerged up to the time of dissection.		
	Less than 4.	4 or 5.	More than 5.
5	2	17	7
6	6	16	18

Two-thirds of all apices with only 5 enclosed initials have 4 or 5 leaves emerged since the winter, and the χ^2 test shows this tendency to be significant

beyond the 0·1 per cent. level. It can be deduced that the majority of stem apices which are reduced to the 'minimal condition' reach the stage of having only 5 enclosed leaf initials after 4 or 5 leaves have emerged in the current season. Only 16 out of 40 of the apices with 6 enclosed initials are at the stage of 4 or 5 emerged leaves, and the χ^2 test shows that the chances are more than 10:1 that the two classes of apex do differ in this respect. Turning now to the April dissections, these apices are not likely to have passed through the minimal condition and returned to the state of 6 enclosed initials already, so that apices with 6 enclosed initials in April can be assumed not to have reached the minimal condition, and so would be expected to have less than 4 leaves

TABLE II

Spring Leaf Initiation and Leaf Emergence under Various Conditions

I. Plants on an exposed hillside overlooking the Bristol Channel.
II. Plants grown in the centre of Cardiff in an open bed.
III. Plants grown in the centre of Cardiff in wooden baskets not plunged in the ground.

A, B, and C, as in Table IV

	I.			II.			III.		
	A.	B.	C.	A.	B.	C.	A.	B.	C.
Jan. 9, 1954	7·6			7·0			6·8		
		∞	∞		∞	∞		∞	∞
Feb. 13, 1954	7·6			6·9			6·8		
		35	16		35	19		52	22
Mar. 26, 1954	6·6			6·0			5·7		
		20	12		15	12		25	14
Apr. 26, 1954	5·6			5·5			4·8		
		14	10		6·1	7		9	11
May 7, 1954	5·4			5·7			5·0		
		7·5	9		16	30		39	54
June 3, 1954	6·6			6·5			5·2		

already emerged; which was found to be the case. Many of the apices dissected in May will have passed through the minimal condition and may have regained the 6 enclosed initials; these will often have more than 5 leaves already emerged, and this number should increase with later dissections—both predictions being confirmed by the observations. Results obtained with forced plants were similar. The assumption that most plants pass through the minimal condition at some time during April or May appears to be justified. The magnitude of the fluctuation and the time of occurrence are fairly consistent from year to year, but Table II shows that there may be appreciable differences between plants grown under different conditions, and probably this accounts for the differences between the results in 1951 and 1952 compared with 1953 and 1954.

The fall in number of enclosed leaf initials from 7 or more in February to the minimal number of 5 in May must be the result of a rate of leaf emergence in excess of the rate of leaf initiation. One reason for this inequality of the two rates can be found in the relation of successive leaf initials to each other in

the dormant apex. During the winter leaf initiation and growth of the younger leaf initials continues although the growth and emergence of the older leaf

Table III

Primordium Length Ratios during the Spring (1953 and 1954)

Leaf initials are lettered according to the order of their emergence in the spring; A is the first leaf to emerge, B the second, &c. The two leaves whose lengths give the primordium length ratio are indicated by their letters. Separate means are given for apices with 5 and 6 enclosed leaf initials.

Date of dissection.	Number of enclosed initials.	Primordium length ratios.						Length of initial (in mm.)	
								Emergent initial.	Youngest primordium.
		A/B	B/C	C/D	D/E	E/F	F/G		
Feb. 17, 1953	7·4	1·7	1·6	2·1	2·9	4·0	13·3	—	0·06
		C/D	D/E	E/F	F/G	G/H			
Mar. 23, 1953	6·6	2·9	1·8	2·3	6·0	4·3		29	0·07
		D/E	E/F	F/G	G/H	H/J			
Apr. 8, 1953	6·0	2·7	1·9	3·5	3·3	5·5		35	0·11
	5·0	2·4	2·8	2·8	4·7	—		25	0·28
		F/G	G/H	H/J	J/K	K/L			
Apr. 30, 1953	6·0	1·7	2·4	2·8	5·2	4·8		29	0·10
	5·0	3·2	2·5	2·7	7·5	—		32	0·20
		G/H	H/J	J/K	K/L	L/M			
May 13, 1953	6·0	2·8	2·5	3·1	5·7	3·8		36	0·08
	5·0	2·8	3·0	5·9	4·7	—		28	0·12
		H/J	J/K	K/L	L/M	M/N			
May 20, 1953	6·0	3·3	2·3	4·0	4·0	4·6		36	0·07
	5·0	3·0	2·3	3·4	5·0	—		33	0·28
Means for April and May 1953:									
6 enclosed initials		2·6	2·3	3·3	4·5	4·5		34	0·09
5 enclosed initials		2·9	2·6	3·7	5·5	—		30	0·22
		A/B	B/C	C/D	D/E	E/F	F/G		
Feb. 13, 1954	7·0	1·6	1·5	2·3	2·7	4·4	5·6	28	0·13
		C/D	D/E	E/F	F/G	G/H			
Mar. 26, 1954	6·0	2·3	2·5	3·0	3·2	4·7		31	0·12
	5·0	3·5	2·8	3·3	5·0	—		28	0·17
		E/F	F/G	G/H	H/J	J/K			
Apr. 26, 1954	6·0	2·2	2·7	4·3	4·0	4·3		31	0·07
	5·0	3·0	3·9	4·7	3·7	—		34	0·16
		F/G	G/H	H/J	J/K	K/L			
May 7, 1954	6·0	2·8	2·8	3·7	3·7	6·0		32	0·05
	5·0	3·2	3·6	4·4	4·3	—		32	0·15
		G/H	H/J	J/K	K/L	L/M			
May 19, 1954	6·0	3·3	2·5	3·5	3·4	4·6		33	0·06
	5·0	3·2	3·7	4·3	3·0	—		29	0·19
		J/K	K/L	L/M	M/N	N/O			
June 3, 1954	6·0	3·5	2·2	2·9	3·7	4·2		38	0·11
	5·0	3·2	3·3	3·0	5·8	—		35	0·19
Means for April and May 1954:									
6 enclosed initials		2·8	2·5	3·5	3·6	4·8		33	0·08
5 enclosed initials		3·2	3·5	—	3·9*	4·3*		32	0·17

* N.B. These values are the means of the youngest and next to youngest primordia, and are placed under the corresponding values for the buds with 6 enclosed initials.

initials is stopped (Arney, 1955). Only the growth of the three oldest leaf initials is affected, the oldest being affected most. As a result, the size ratios between the three oldest leaf initials are decreased (see Table III) as the younger

initials catch up with the older ones. On the assumption that the acceleration of growth and resumption of activity in the spring occurs simultaneously and equally in the stem apex and in the young and old leaf initials, one would expect the three oldest leaf initials to emerge in close succession because their size differences have been telescoped by differential winter growth. This would lead to a reduction in number of enclosed leaf initials after early spring growth, but could not, by itself, reduce the number of enclosed initials below the number present in the autumn before inhibition of emergence began to take effect. The observed phenomena are in accord with this assumption, for the number of enclosed initials falls to 6 at the end of March or beginning of April after the first 3 leaves have been produced; the subnormal number of only 5 enclosed initials is not reached until a later stage after 4 or 5 leaves have emerged. It is therefore clear that the telescoping of the sizes of the oldest leaf initials as a result of winter growth only explains part of the fall in number of enclosed leaf initials during the spring, and some other cause must be sought for the remainder of the effect.

Since the 'minimum condition' occurs after only 4 or 5 spring leaves have emerged, it is clear that 2 or even 3 of the initials remaining enclosed at this stage were initiated before the end of the winter, and at least 1, possibly 2, before the incidence of dormancy in the autumn. Hence the 'minimum condition' is not related to any discontinuity between the leaf initials produced before or during the winter and those produced after the resumption of spring growth.

If the stipules enclosing the stem apex in May were much smaller than at other times of the year, the leaf initials emerging in May would exceed the length of the enclosing stipules and emerge at an earlier stage of development than usual; with sufficient difference in size of stipules this emergence could occur before the initiation of the sixth youngest leaf initial at the stem apex. But Table III shows that the leaves emerging in May are almost exactly as long at emergence as those emerging in March and in June. A difference of over 30 per cent. would be required to enable emergence before the sixth primordium is initiated, so the drop in number of enclosed leaf initials in April and May cannot be caused by differences in size of initials at emergence.

Apart from difference in size at emergence, changes in the number of enclosed initials can only be brought about by a difference between leaf initiation rate and leaf emergence rate during the period of change. The drop in number of enclosed initials in April and May could be the result of either a very slight excess of leaf emergence rate over leaf initiation rate maintained over a period of weeks or of a sudden and temporary change—either a temporary cessation of leaf initiation or a temporary acceleration of the elongation growth of the older leaf initials leading to a spurt in leaf emergence rate. If this sudden change occurred on different dates in different individual plants, the random samples used for dissection could produce mean values giving a smooth drift of the kind actually observed (Table I). The occurrence of a

more or less sudden and temporary change in leaf initiation rate or in the growth rate of the leaf initials which is not reflected in both simultaneously can be ruled out. Not only does no temporary increase in leaf emergence rate occur consistently at this time of the year, but also the evenness and consistency of the primordium length ratios in Table III show that no sudden and independent fluctuations of either leaf initiation rate or growth rate of the leaf initials have occurred. The value of the primordium length ratio as an indication of the relation between leaf initiation rate and the elongation growth of the leaf initials has been explained in an earlier study (Arney, 1953).

There is very little difference between the primordium length ratios of apices with different numbers of enclosed initials during April and May, but the primordium length ratios of all apices dissected at this time are greater than those for apices dissected at other periods of active growth, thus confirming the higher growth rate of the leaf initials compared with the leaf initiation rate during the spring. Although the primordium length ratios for apices with only 5 enclosed initials during April and May are appreciably higher than the corresponding values for apices with 6 enclosed initials during these months in both 1953 and 1954, the difference does not occur consistently on all dissection dates, and is not nearly great enough to explain the presence of the extra leaf initial in the one set of apices. The mean lengths of the youngest primordium given in Table III show that the apices with 6 enclosed initials have only just initiated the sixth primordium, while the apices with only 5 enclosed initials are just on the point of initiating the sixth primordium. There is nothing like a whole plastochron difference between the two. This is the factor which plays the greater part in bringing about the difference in number of enclosed initials, and not the difference in primordium length ratios.

Since the primordium length ratios indicate the balance of leaf emergence rate and leaf initiation rate, the fact that there is so little difference between the ratios for apices with 5 and with 6 enclosed initials shows that the balance of leaf emergence and initiation is much the same in both, and this is additional strong evidence against the postulation that only a few apices ever pass through the minimal condition during the spring, and that many apices with 6 enclosed initials do not experience the lag of leaf initiation on leaf emergence which brings about the minimum condition.

From records of the leaves produced in winter and spring by the plants which were subsequently dissected it is possible to determine the total number of leaves initiated between the beginning of December and the various dates on which dissections were carried out. It is assumed that all the plants dissected on each occasion were comparable at the beginning of December, and that the mean number of enclosed primordia at the beginning of December for each batch of plants would be 6·5 in 1953 and 7·0 in 1954 (Arney, 1955). The rates of leaf initiation can be calculated from the difference between the total number of leaves initiated since December for each successive occasion on which dissections were performed. Calculated values for the plastochron interval for initiation are compared with the observed plastochron interval for

emergence in Table IV. The expected lag of leaf initiation rate on leaf emergence rate up to the end of April is clearly shown. In both seasons the leaf initiation rate increases slowly from the very low winter rate and reaches a very high peak value corresponding to a plastochron interval of 5–6 days at the end of May. This suggests a slow recovery from the dormant condition rather than a sudden leap into active growth on the part of the apical meristem, and this contrasts with the rather more sudden resumption of activity by the older leaf initials. The picture of a gradual adjustment of the balance of leaf initiation rate and leaf emergence rate from the early spring condition in

TABLE IV

Rate of Leaf Initiation and Rate of Leaf Emergence during the Spring

A. Number of enclosed leaf initials.
B. Plastochron interval (in days) based on leaf initiation.
C. Plastochron interval (in days) based on leaf emergence.

	1953				1954*		
	A.	B.	C.		A.	B.	C.
Jan. 17	7·0			Jan. 9	7·6		
		78					
Feb. 17	7·4			Feb. 13	7·6		
		30	15				
Mar. 10	7·0					35	16
		22	16				
Mar. 23	6·6			Mar. 27	6·6		
		21	13				
Apr. 8	6·1					20	12
		23	12				
Apr. 30	5·9			Apr. 26	5·6		
		9·3	9·7			14	10
May 13	5·6			May 7	5·4		
		5·5	11·6			7·5	9
May 25	6·2			May 19	5·7		
						6	9
				June 3	6·6		
						10	8·5
				June 17	6·4		

* N.B. Values for only one of the three sets of plants represented in the means in Table I.

which the latter exceeds the former, confirms the conclusions reached in previous paragraphs. The further explanation of these peculiarities of spring growth must be sought in terms of the cause of the comparatively low leaf initiation rate during March and April. The two possibilities which most readily suggest themselves are recovery from dormancy, and competition from the developing inflorescences. Both alternatives have been explored experimentally and will be considered in the following sections.

LEAF PRODUCTION RATES IN FORCED PLANTS

The behaviour of plants during forcing varies according to the growing conditions used for forcing, but also depends very much on the date at which

forcing commences and on the previous treatment of the plants. The complicated situation revealed by forcing experiments is still under investigation, and in this section attention will be confined to those experiments which provide an answer to the questions: Does abnormally early resumption of active growth and leaf emergence accelerate the onset of the 'minimal condition'? and What is the effect of applying long days and warm conditions during the first 2 months of spring growth in this connexion?

It has already been mentioned that, as in the resumption of growth under

TABLE V

Leaf Emergence and Leaf Initiation in Forced Plants

Plants were forced in 18 hours light in a greenhouse at a mean day temperature of 65° F., and a mean night temperature of 50° F.
Columns A, B, and C are as in Table IV.

Date of dissection.	Plants forced on Jan. 29, 1953. A.	B.	C.	Plants forced on Feb. 9, 1953. A.	B.	C.
Jan. 29	—					
		—	9			
Feb. 9	—			—		
		—	11		—	6·5
Feb. 22	6·7			6·3		
		22	12		13	8
Mar. 16	5·5			5·2		
		9	12		8	11
Apr. 6	5·2			5·9		
		9	11			
May 1	5·8					
		12	13			
May 25	6·0					
Time from the start of forcing to the minimal condition (only 5 enclosed initials)		55 days			35 days	
Time from the emergence of first inflorescence to minimal condition		30 days			20 days	

normal spring conditions, the first three leaves emerge in rapid succession on forced plants. This occurs in all forcing experiments which succeed in bringing about the resumption of active growth, and column C in Table V contains results which are typical.

Table V shows that the forced plants undergo a change in the number of enclosed initials which is the same as the normal spring behaviour. Leaf *initiation* rate increases slowly to its maximum, and then decreases again to the normal steady rate, while leaf *emergence* starts at an abnormally high rate and falls to the normal rate. The growth rate of the earlier forced plants is rather slower than that of the later forced plants, and both are higher than that of plants growing out of doors in the spring. The time elapsing between the resumption of active growth and the achievement of the 'minimal condition' is shorter with the higher growth rates, and also occurs sooner after the

emergence of the first inflorescence when the plants are growing faster. The leaf initiation rate increases to its maximum more quickly under forcing conditions, and all growth processes seem to be accelerated to supra-normal rates, including the change in the balance of leaf initiation rate and leaf emergence rate. There does not appear to be any differential effect of forcing conditions on leaf initiation compared with leaf emergence, for in such a case one would expect a different number of enclosed initials at the 'minimal condition'—a lower number of enclosed initials indicating a greater acceleration of leaf emergence than of leaf initiation, and vice versa.

The February forced plants show a very marked tendency to reach the 'minimal condition' after the emergence of the 4th or 5th leaf, and so behave in the same way as the normal plants. The January forced plants appear to reach the 'minimal condition' only after 6 or 7 leaves have emerged, but the number of observations is insufficient to establish this apparent difference. On the whole, therefore, the forced plants show the same changes in growth rate of apex and leaf initials as the normal plants; leaf initiation rate recovers only slowly from winter dormancy, while leaf emergence starts with an immediate spurt which later dies away. The minimal condition is reached earlier in time but at the same growth stage as in the normal plants, and is therefore unconnected with any particular climatic or environmental factors and independent of the absolute growth rate of the plants. The phenomenon is clearly connected with some internal factor which is operative only at a certain stage in the seasonal growth cycle.

Competition from the Growth of the Inflorescence

In 1953 a number of normal plants was selected at random in the beds and labelled for defloration. The inflorescences were dissected away from these plants as soon as they emerged from the stipules covering the stem apex (when the whole inflorescence was about 30 mm. long). These deflorated plants were dissected at intervals early in May, when the control plants were expected to be reaching the minimal condition of only 5 enclosed leaf initials. The control plants showed only 1 plant with less than 6 enclosed initials out of the 7 dissected on April 30, while 3 plants out of the 11 dissected on May 13 had less than 6 enclosed initials. The deflorated plants were dissected between May 2 and May 7 and showed no plants with less than 6 enclosed initials, and 11 plants with 6 enclosed initials and 5 plants with 7 enclosed initials. None of the control plants dissected on the two dates mentioned had 7 enclosed initials. This result seems to show that the post-emergence development of the inflorescence has some effect in reducing the rate of leaf initiation.

In 1954 some confirmation of this conclusion was forthcoming from a very different type of experiment. On September 1, 1954, a number of young runner plants growing in fruit baskets were transferred to long-day treatment by moving them into a lighted and warmed greenhouse every evening. This treatment did not stop inflorescence initiation in all plants, but 13 out of 40 plants produced no inflorescences, or had only just initiated a single

inflorescence at the time of dissection, when the other plants under the same treatment had inflorescences already matured and fruiting. The results of dissections of these two groups of plants, both growing under exactly the same conditions, are summarized in Table VI, which makes it very clear that the minimal condition only occurs in those plants on which inflorescences have emerged and are approaching the blossom period. The presence of one or more unemerged inflorescence initials within the apex appears to have no effect at all in reducing the number of enclosed leaf initials. The occurrence of the minimal condition in these plants, which have not been allowed to enter dormancy, appears to indicate that the slow recovery of the leaf initiation

TABLE VI

The Number of Enclosed Initials in Flowering and Non-Flowering Plants

The plants had been kept in long days and warm nights since September 1 and this postponed inflorescence initiation in about 50 per cent. of the plants.

	Plants already flowering			Plants without any inflorescences emerged		
	Mean number of initials.	Number of plants with		Number of plants with		Mean number of initials.
		5 initials.	6 or more initials.	5 initials.	6 or more initials.	
Nov. 30	5·3	6	3	0	8	6·4
Dec. 5	5·8	2	2	0	3	6·3
Jan. 5–13	6·0	1	7*	1	5	5·8

* N.B. Five of the 7 flowering plants with 6 enclosed initials on this date had flowered at least 2 months previously, and had no younger emerged inflorescences.

rate from the dormant level is not necessarily the cause of the minimal condition.

These results, together with the defloration experiments, are strong evidence for associating the occurrence of the minimal condition with the post-emergence development of the inflorescence. But it must be remembered that normal plants recover from the minimal condition and regain the normal number of enclosed initials by the end of May, while the second inflorescence is still developing and flowering. Also the plants dissected on January 5 (Table VI) all had at least 6 enclosed initials even though many of them had inflorescences just recently emerged and with flowers just opening. In both these cases plants which had been flowering for some time showed no tendency towards the minimal condition during the development of the *second* inflorescence. The effect therefore is probably not the result of a straightforward competition between growing inflorescence and apex.

CONCLUSIONS

1. The special relationship of the three oldest leaf initials enclosed in the stem apex at the end of the winter leads to a spurt in leaf emergence at the recommencement of spring activity.

2. The rapid initial rate of leaf emergence in early spring contrasts with the slow increase in leaf initiation rate from the very low dormant rate. The resulting lag of leaf initiation rate on leaf emergence rate during early spring leads to a reduction in the number of enclosed leaf initials. The number of enclosed leaf initials, which has been increased as a result of winter growth activity, is restored to normal by the rapid emergence of the three oldest leaf initials which have all reached much the same stage of maturity as a result of winter growth.

3. The observed reduction in number of enclosed leaf initials is greater than would be expected from the restitution of winter growth anomalies by rapid emergence of the older leaf initials. Evidence is available that almost all stem apices pass through a 'minimal condition' containing only 5 leaf initials enclosed at the apex—1 less than the normal number. This further fall in the number of enclosed leaf initials is also shown to be due to a lag of leaf initiation rate on leaf emergence rate, which is not connected with climatic or environmental factors, but seems likely to be connected in some way with the post-emergence development of the first inflorescence.

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